ADSTEC

User's Manual

Ver. 1.6

April 2015

ADSTEC Corporation

Introduction

Thank you for purchasing ADSTEC Corporation “Metaphase Harvester **HANABI-PI**”.
We hope you will remain a satisfied customer for years to come. Chromosome specimen maker Metaphase Harvester **HANABI-PI** does the processing of cell harvesting from the cultured cell automatically, simplifying a complicated process and producing uniform cells. The main operation of Metaphase Harvester is the constant temperature centrifugal separator structure, a hypotonic treatment using pre-heated hypotonic solution that enable fixation processing with cooled fixative solution. The centrifugal separator section is located at the center with 4 sets of centrifugal sections, 4 tubes per set, capable of producing 16 specimens at one time by injecting solutions and aspiration solutions. By using the **HANABI-PI**, a large amount of stable chromosome specimens can be produced enabling cell harvesting.

Metaphase Harvester HANABI-PI
EU model

- Approved conditions of use

The following conditions of use and warranty contents shall be applied when operating our "Metaphase Harvester **HANABI-PI**" (hereinafter called "the equipment") according to this manual unless otherwise specified in the contract or specifications.

- Warranty contents

Warranty period

The warranty period of the equipment shall be for one year, from the date of original purchase or delivery to a specified site.

Scope of warranty

In the event of any failure occurring due to manufacturing defect during the above warranty period, ADSTEC Corporation will replace or repair defective parts at no charge at the place of purchase. However, defects occurring under the following circumstances shall not be covered:

a) When this equipment is used under conditions or in an environment or manner, or for a purpose other than that specified in this manual or specifications.
b) When the defect is caused by something other than the equipment itself.
c) When the equipment is modified or repaired by anyone other than our company.
d) When the equipment is used for a purpose other than that intended.
e) When the cause thereof was impossible to predict given the level of science and technology prevailing at the time of equipment delivery.
f) When the defect is due to other causes for which we are not responsible, such as accidents or natural disaster.

This warranty applies only to the equipment, and does not cover other damages induced by the failure of this equipment.

- Limitation of liability

(1) ADSTEC Corporation shall not be liable for any consequential or incidental damage caused by the equipment.
(2) Concerning programmable-type products among the equipment, we shall not be liable for programs created by anyone other than our company, and any result caused by such programs.

- Conditions for appropriate use

(1) Do not use the equipment for any use other than that specified in the specifications. When using the equipment in combination with other systems, the user must be responsible for checking compatibility between the equipment and the other system, machine, or equipment to be used. We accept no liability for the compatibility of this equipment if this is not done.
(2) The example applications presented in this manual are for reference purposes only. Be sure to check the functions and safety of the equipment before operation.
(3) Ensure you fully understand all the prohibitions and precautions before use and strictly follow them during operation, in order to avoid the risk of unexpected damage to you or any third party that may be caused by improper handling of the equipment.

- Change in specifications

The specifications and accessory lineup of the equipment described in this manual are subject to change without prior notice, when required for product improvement or other reasons.

- Scope of application

The above contents apply to transactions and use of the equipment in Japan.
Please contact our Sales Department for transactions or use elsewhere.

Safety Precautions

Indications and meanings for ensuring safety during use of equipment

Proper handling and checking are essential to ensure the equipment is used safely.
This manual indicates items that may result in injury or death when the equipment is handled improperly, at three levels, namely DANGER, WARNING, and CAUTION.

- Explanation of sign

 **DANGER**	Indicates that, if this sign is ignored and the equipment improperly handled, it may lead to imminent danger of death or severe injury.
 **WARNING**	Indicates that, if this sign is ignored and the equipment improperly handled, it might lead to death or serious injury, or minor injury or physical damage.
 **CAUTION**	Indicates that, if this sign is ignored and the equipment improperly handled, it might lead to minor injury or physical damage, although it is unlikely to result in serious injury.

- "Serious injury" above refers to loss of sight, injury, burns, electric shock, and bone fractures associated with aftereffects or requiring hospitalization or long-term stay in hospital.
- "Minor injury" refers to injury, burns, or electric shock that are not included in the serious injuries category.
- "Physical damage" refers to extended damage to facilities or property.

The "triangle" sign is used to warn (or caution) the user about dangers (or dangerous conditions) that may occur due to apparently improper operation or use of the equipment.

In addition, the following signs are also used in this manual and on the equipment surface.
All these signs contain important information and must be followed at all times.

	RISK OF ELECTRICAL SHOCK Risk of electric shock under particular circumstances.
	CAUTION: HOT Risk of burns if this location is touched.
	REVOLVING BODY
	General prohibitions These signs indicate prohibited actions.
	DO NOT TOUCH
	GROUNDING REQUIRED If a safety ground terminal is provided, be sure to ground the ground lead.
	General compulsory instructions This sign indicates that the item in question requires attention.

Never peel off the WARNING labels attached to the product.

Important Safety Precautions

WARNING

(1) Always follow the directives specified in this manual when using the equipment. Do not try to operate or handle the equipment in a manner other than that specified in this manual. Do not replace any parts with other units of differing specifications, or otherwise modify the equipment. Do not use the equipment for any purpose other than that specified in this manual. Doing so may result in mechanical failure or personal injury.
(2) Keep the equipment away from fire. Do not modify it in any way.
(3) Do not use the equipment in a place where it may get wet.
(4) Be sure to ground the equipment to prevent the risk of electric shock through current leakage.
(5) In the event that any abnormal sound, smell or any other abnormal conditions are detected during use of the equipment, immediately stop operation and contact the distributor or our company.

CAUTION

(1) The equipment is intended for use in the U.S. and re-export is not permitted.
(2) The user of this equipment must take training in the beginning.
(3) Periodical maintenance is vital to ensure the equipment can be used safely for an extended period. Please execute a periodic check once to this equipment in one year. The manufacturer or the professional skill person in the agency maintains the equipment. The maintenance contract is separately necessary.
(4) We do not accept any liability, other than for equipment repair, for any damage caused by equipment failure.
(5) Ensure the equipment is always operated according to the order and method specified in this manual. Failure to do so may result in the equipment malfunctioning or failing.
(6) Only specially trained personnel should remove or replace parts.
(7) Do not cover the openings used for ventilation (air vents on the side and bottom of the equipment).
(8) Before connecting the power cable or checking the equipment, ensure it is fully switched off. Before checking the equipment, also unplug the cable from the socket.
(9) Please remove equipment inlet side of the power cable in the emergency (smoking and ignition, etc.).
(10) To completely stop the equipment, turn the power switch on the front side of the equipment OFF and then unplug the cable from the socket.
(11) Separate the power supply code from the Inlet of the main body of the equipment at once in the emergency (smoking and ignition, etc.).
(12) Please wipe off with the paper towel etc. when you spill the reagent or the sample in the equipment. Install the rubber glove for the set of the reagent and the sample and taking out the sample. Please flush it at once in water when the reagent sticks to clothes and the skin.
(13) Please clean it with the paper towel etc. to contain the ethanol when dirt adheres to the plastic part and the metal part of the equipment. Or, please contain water and use the towel etc. wrung hard. Do not use an organic solvent. The transformation might discolor plastic.
(14) The expected lifespan of the equipment is five years and it should be replaced when this period has passed.
(15) Do not disclose any of the contents of this manual, in whole or in part, to any third party without the prior consent of ADSTEC Corporation.
(16) The contents of this manual are subject to change without prior notice at any time, due to improvement of equipment specifications or other reasons.
(17) If you have any query or lack of understanding regarding any of the contents of this manual, please contact the distributor or our Sales Department.

Chemical information:

The hypotonic solution: 0.075 M KCl is used.
Fixative solution: Acetic acid and methanol mixture ratio 1:3.

Acetic acid (CH3COOH):
Physical properties
mp(℃): 16.7
bp(℃): 118
Specific gravity (20/4℃): 1.049
Flash point (℃) 39 (closed cup Merck)

Methanol CH3OH:
Physical properties
mp(℃): -97.8
bp(℃): 64.7
Specific gravity (20/4℃): 0.7915
Flash point (℃) 11℃ and explosion range: 6.7-36.5v/v%
Additionally, the methanol is mixed with water at an arbitrary rate.

CAUTION
When the acetic acid and the methanol are mixed, it does without fail in the place where the ventilation equipment of the draft chamber etc. is done. An acetic acid, a methanol, and a fixed liquid are dangerous. These are drunk and no suction. Do the great caution for handling.

【 measures for safety 】

- Do not handle the safety attention concerning the acetic acid and the methanol until reading and understanding.
- Obtain the manual concerning the acetic acid and the methanol before it uses it.
- Do not eat and drink or do not smoke while using the acetic acid and the methanol.
- Keep away from the ignition source like the one of heat, the spark, the naked flame, and the high temperature. No smoking.
- Use an explosion-proof type electric equipment, the ventilator, and the lighting. Prevent the ignition by the static discharge and the spark.
- Avoid exposing with a personal protection tool and a ventilator.
- Wear the protective glove, protective glasses, and the protection mask.
- Use it only in the place where ventilation is good.
- Neither mist, steam nor the spray are inhaled.
- Wash your hand well after handling.

Installation Precautions

To ensure extended use of the equipment

(1) Before unpacking, transfer the equipment to a flat area close to the installation site.
(2) After unpacking, ensure that the following items are all included:

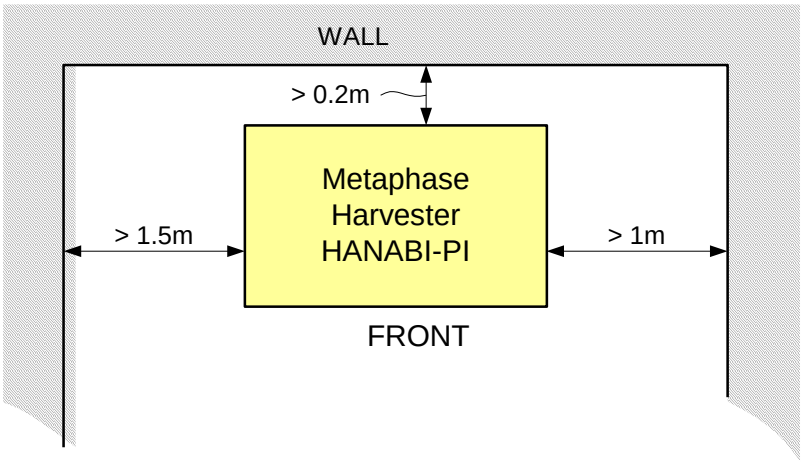
HANABI-PI Attachment Items

No.	Item descriptions	Qty(s)
1	HANABI-PI main body	1
2	10 Liter Distilled Water Carboy	1
3	10 Liter Waste ‘A’ Carboy	1
4	10 Liter Waste ‘B’ Carboy	1
5	Power Cord	1
6	Cooling Box (styrene foam)	2
7	500ml PBS Bottle, PP w/cap & fitting	1
8	500ml Hypotonic Bottle, PP w/cap & fitting	1
9	500ml Water Bottle, PP w/cap	2
10	500ml Fixative Bottle, PP/Brown w/cap & fitting	1
11	500ml Fixative Bottle, PP/Brown w/cap	1
12	500ml Water Bottle, PP/Clear w/cap	1
13	Users Manual	1
14	Maintenance Manual	1
15	Power Interlock Door Key	1
16	500 Falcon Tubes (50 x 10 = 500pcs)	1
17	Silicon rubber for tube holder (16pcs)	1
18	Reagent Bottles Shelf	1
19	Bottles Tray	1
20	Metric hex-head wrench set	1

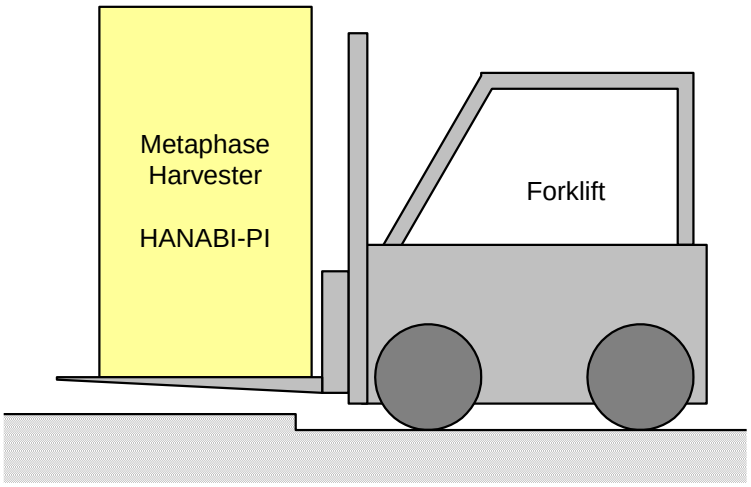
Also ensure that the product and accessories are free from any damage or deformation.

(3) Choose an appropriate location for installation and ensure the equipment is installed on a level surface. The following sites in particular should be avoided:

- Exposed to high temperature and humidity
- Exposed to strong vibration or impact
- Potentially exposed to salt content or corrosive gas
- Inclined (uneven) areas
- Areas in the vicinity of radio equipment (which may result in the equipment to malfunction)
- Dusty areas
- Confined spaces (The equipment requires space around the exhaust outlets to enable air cooling.)



(4) Please take space in left (>1.5m), right (>1.0m), and back (>0.2m) from the wall side in this equipment.



(5) Please move the equipment by at least three people or more. Please push four corners when you move by handling the caster of the equipment. The cover might dent when the cover is pushed. Please move safely carefully by the use of the forklift etc. when you move, as there are a difference and a distance. The forklift must penetrate two forks forward under the equipment backward (Or, from the rear side forward).

(6) Method of connecting safety earth and power supply. Please connect the safety earth by the following method for the prevention of the electric shock.

-The safety earth is connected if the power plug is connected when power supply outlets are three poles.
-The conversion plug (three pole →2 pole) is used when power supply outlets are two poles. The earth cable of the conversion plug is connected with the earth terminal in the building.

(7) This equipment is only for the indoor use. The equipment cannot be used in outdoor.

Operating Precautions

(1) Ensure more than 30 minutes are allowed for the preliminary run.
(2) Never turn the equipment OFF during the centrifuging operation. If you do so, the centrifuge will stop suddenly, which may result in damage to samples and serious damage to the equipment.
(3) Never open the door during operation (main process).
(4) Please turn on the FAN switch in the equipment in which safety filter (Microbiological Safety Filter) is installed and use it.

Users Manual Revision History

The manual revision symbols are listed on the last page of this manual.

Manual number: HANABI-PI Version 1.6 April 20, 2015

Revision symbol	Revision date	Revised page / Contents
V1.1	Nov 01, 2010	Item modification
V1.1a	May 25, 2011	Address change of the corporation
V1.2	Sep 22, 2011	Page 40, corrected re-start 123-ABC
V1.3	Feb 13, 2013	Revised 3.27 SUB
V1.4	Mar 7, 2013	Revised procedure figure and formula notation of the processing execution time
V1.4a	Sep 26, 2013	Page 5, revision of procedure
V1.4b	Dec 2, 2013	Page 8, adding aspiration level setting and reagent injectable range
V1.5	Aug 21, 2014	Add doing Drainage work into “2.4. Procedures” Revise a chart at “5.2. Operation Check Sheet”
V1.6	April 20, 2015	Revisio of company’s logo

Metaphase Harvester HANABI-PI User's Manual

Table of Contents

1. Explanation of the terms

Main process: A series of operations executed for the purpose of harvesting. It is divided into 12 processes (from A to L).

Protocol: Indicates the contents (order and conditions) when carrying out the main process.

Reagent exchange: An operation to exchange the solution in the tube on the reagent line (for hypotonic and fixative solutions).

Needle washing: An operation to wash the inner and outer wall of the aspiration needle with washing solution (acid or alkaline, etc.)

Station: When the main process is executed, 4 tubes A, B, C and D are processed as a single group, and each group is called a “station.”

Positioning: Indicates support for each station at the time of sample setting, reagent injection, aspiration, and removal, to ensure the station remains immobile.

Setting position: Indicates the front position in which a sample tube will be set.

Aspiration position: Indicates the position in which waste solution will be discharged.

Injection position: Indicates the position in which solution will be injected and below which a stirring shaft is provided.

Pitch: Indicates a clockwise rotation by 90 degrees at a time in order of A, B, C and D to be carried out at the time of injection.

Positioning completion lamp: Indicates that each station is supported safely for injection or removal. It is located on the inner wall on the front side of the equipment.

Origin: Indicates the status where the aspiration needle is in the washing position, Station #1 is in the setting position.

Select Function screen: A screen to choose whether to execute the main process or input the setting value for processes related to the main process

Turntable: Indicates the table that supports 8 stations and upon which the entire process, from start to completion, will be carried out.

- Low speed: Indicates the speed for positioning.
- High speed: Indicates the speed for centrifuging.

Alarm sound (Meaning)

- When the positioning is completed for setting or removal
- At the start of the main process
- When the main process is completed: 1-second cycle (intermittent sound)
- Following the stop: 1-second cycle (intermittent sound) at a high tone.
- Indicating a caution: 1-second cycle (intermittent sound) at a high tone.

Stop switch: If this is pressed during operation, the operation will stop instantly. To release the stop switch, turn it clockwise. (If the switch is pressed while the table is rotating at high speed, the table rotation will gradually slow down until it stops.)

2. Operational procedures

2.1. Operating part names

(1) Operation panel
The operation panel includes display and touch switch functions.

(2) STOP switch
The switch is for the stop. Pressing this button stops the operation of the equipment.

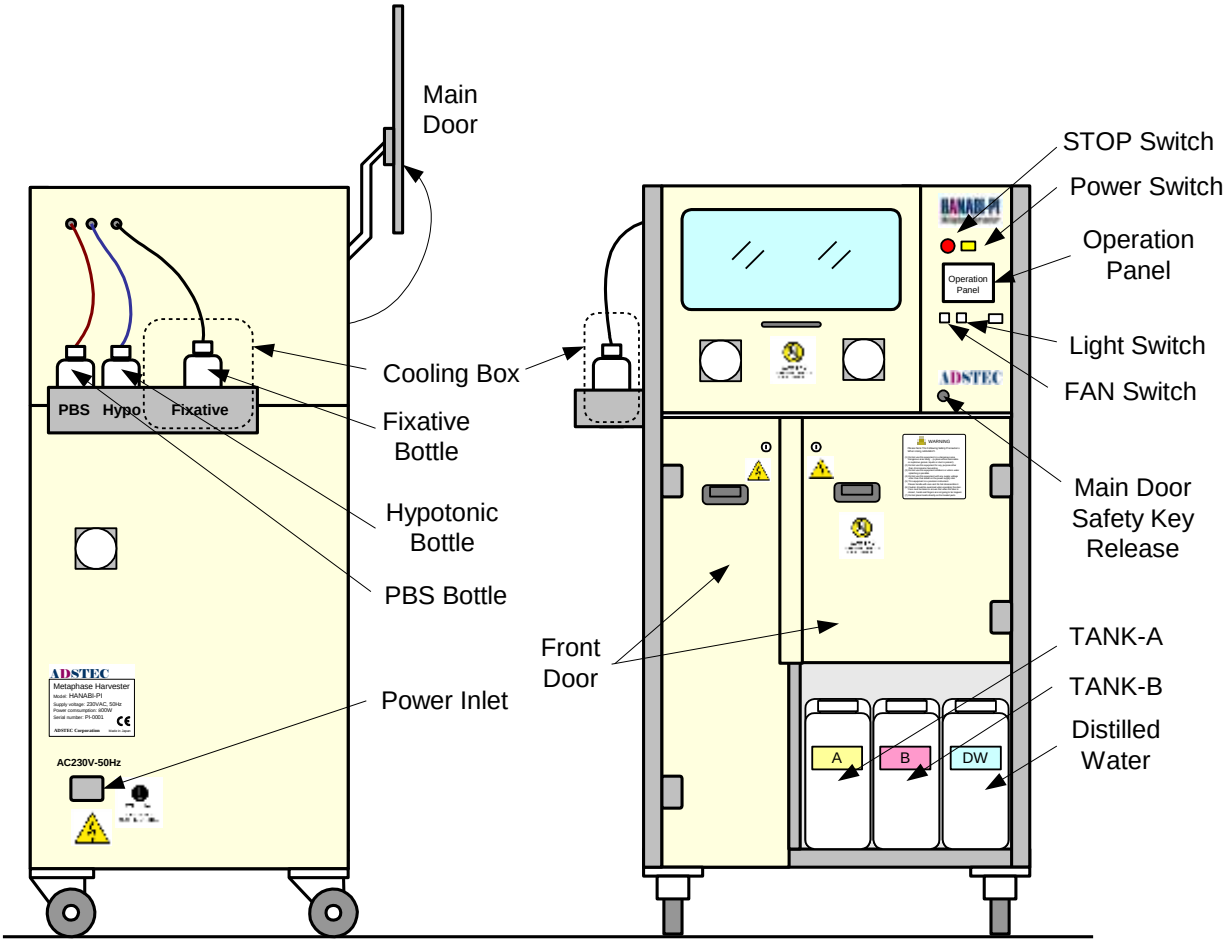
(3) Light switch
The switch illuminates the inside of the upper door of the equipment.

(4) Power switch
The switch is used to turn the main power ON or OFF.
The equipment will activate about 30 seconds after the switch is turned on.

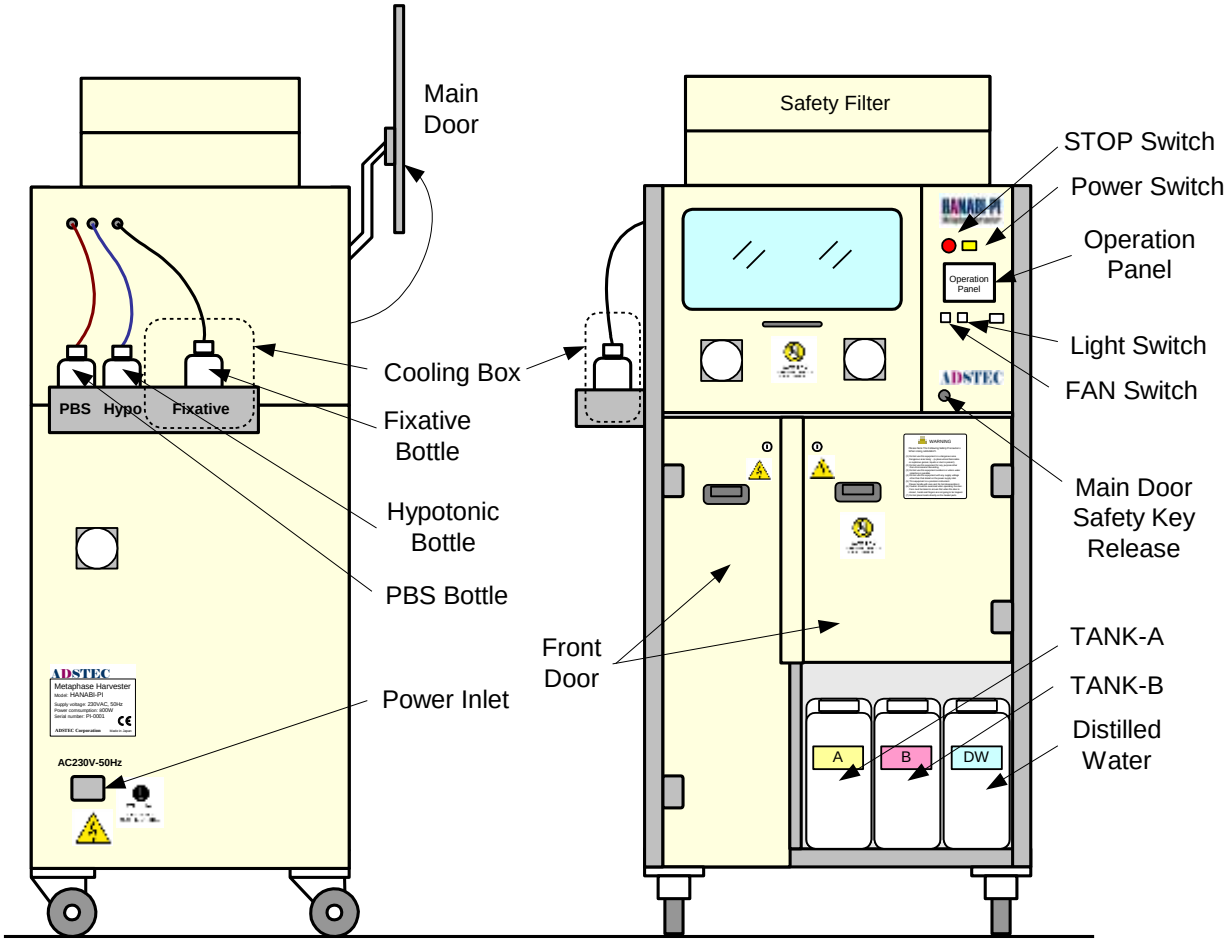
(5) FAN motor switch
It is mounted on EU specification equipment, and FAN for Microbiological safety filter is turned on and off.

2.2. Warning indication

On the surface of the equipment, warning indications are provided at the points shown in the figure.



EU specification (Microbiological safety filter mounting)



2.3. Preparation

The following items should be prepared:

a) Distilled water (ion-exchange water): 5 liters or more
b) Hypotonic solution / PBS (If you use it.)
c) Fixative solution
d) Sample

(1) Reagent preparation volume

See the following "How to Calculate the Reagent Preparation Volume" for the reagent volume. The values shown there are only for design purposes.
Be sure to check the remaining solution volume using each protocol.

PBS / Hypotonic Solutions

ABC	No.	Items	Vol. [ml]	Proc. Times	Number of Samples			
					4	8	12	16
1	1	Reagent exchange	40	1	40	40	40	40
	2	Injection	5	1	20	40	60	80
	3	Refresh injection line	25	1	25	25	25	25
	4	Amount of reserve	50	1	50	50	50	50
	Total volume [ml]				**139**	**163**	**187**	**211**
2	1	Reagent exchange	40	1	40	40	40	40
	2	Injection	5	2	40	80	120	160
	3	Refresh injection line	25	2	50	50	50	50
	4	Amount of reserve	50	1	50	50	50	50
	Total volume [ml]				**180**	**220**	**260**	**300**
3	1	Reagent exchange	40	1	40	40	40	40
	2	Injection	5	3	60	120	180	240
	3	Refresh injection line	25	3	75	75	75	75
	4	Amount of reserve	50	1	50	50	50	50
	Total volume [ml]				**225**	**285**	**345**	**405**

Fixative Solutions

IJK	No.	Items	Vol. [ml]	Proc. Times	Number of Samples			
					4	8	12	16
1	1	Reagent exchange	30	1	30	30	30	30
	2	G process Injection	0.5	1	2	4	6	8
	3	Injection	5	1	20	40	60	80
	4	Refresh injection line	12	1	12	12	12	12
	5	Amount of reserve	50	1	50	50	50	50
	Total volume [ml]				**118**	**144**	**170**	**196**
2	1	Reagent exchange	30	1	30	30	30	30
	2	G process Injection	0.5	1	2	4	6	8
	3	Injection	5	2	40	80	120	160
	4	Refresh injection line	12	2	24	24	24	24
	5	Amount of reserve	50	1	50	50	50	50
	Total volume [ml]				**146**	**188**	**230**	**272**
3	1	Reagent exchange	30	1	30	30	30	30
	2	G process Injection	0.5	1	2	4	6	8
	3	Injection	5	3	60	120	180	240
	4	Refresh injection line	12	3	36	36	36	36
	5	Amount of reserve	50	1	50	50	50	50
	Total volume [ml]				**178**	**240**	**302**	**364**
4	1	Reagent exchange	30	1	30	30	30	30
	2	G process Injection	0.5	1	2	4	6	8
	3	Injection	5	4	80	160	240	320
	4	Refresh injection line	12	4	48	48	48	48
	5	Amount of reserve	50	1	50	50	50	50
	Total volume [ml]				**210**	**292**	**374**	**456**
5	1	Reagent exchange	30	1	30	30	30	30
	2	G process Injection	0.5	1	2	4	6	8
	3	Injection	5	5	100	200	300	400
	4	Refresh injection line	12	5	60	60	60	60
	5	Amount of reserve	50	1	50	50	50	50
	Total volume [ml]				**242**	**344**	**446**	**548**

2.4. Procedures

The procedures are as follows:

(1) Turn the main power ON, then prepare the reagent (Hypotonic, PBS and Fixative solutions).

(2) The equipment will then carry out a preliminary run for about 30 minutes.

Note : When "Bath and Preheat OFF" is set to the last execution protocol, warm up cannot be done. Please execute the setting change first of all when you change the protocol.

(3) Execute the reagent exchange on the operation screen.

(4) Select a protocol. Select “Main Process” on the SELECT FUNCTION screen of the protocol selected above. To change items, select “Set Items” on the SELECT FUNCTION screen. After setting the desired items, select “Main Process”.

(5) Next, select the set sample number.

(6) Set the sample tubes on the SET CONDITION screen.

(7) Select “MAIN START.” (Press and hold the button.)

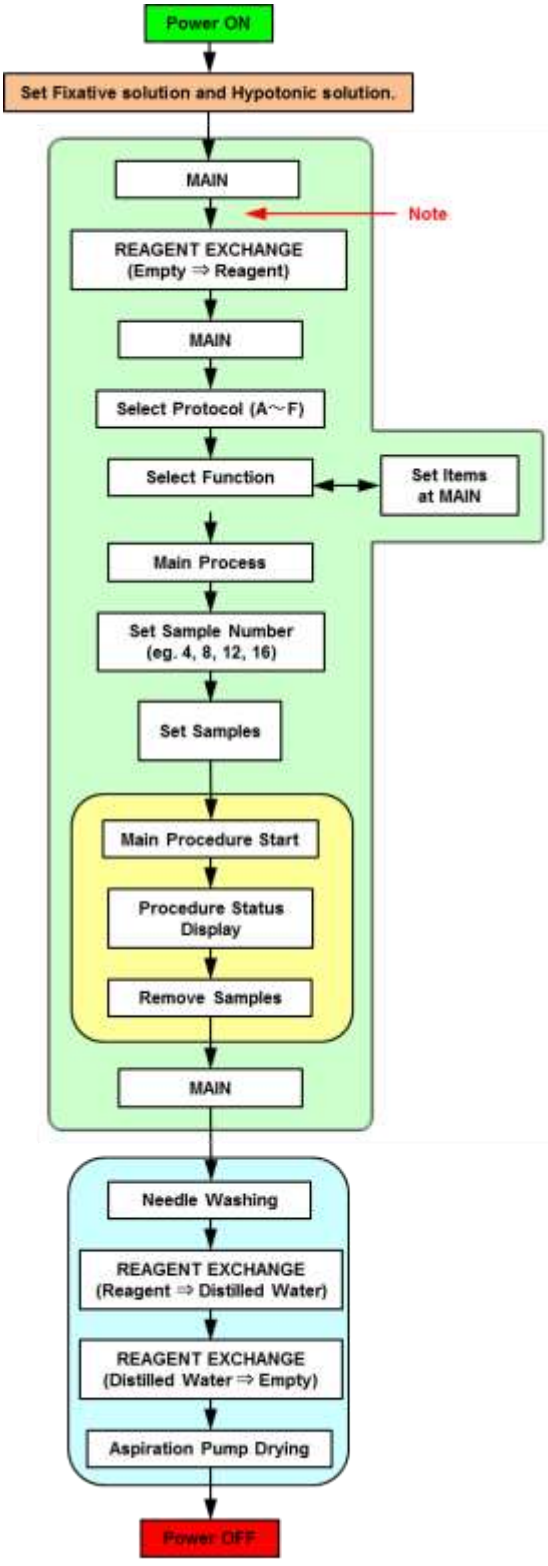
(8) Centrifuging will start and the procedure status will be displayed until the REMOVE SAMPLES screen appears.

(9) Remove the harvested samples on the REMOVE SAMPLES screen.

(10)Press COMPLETE button to return to the MAIN screen.

(11)To continue harvesting, first check the reagent volume.
Then proceed to the SELECT PROTOCOL, SELECT FUNCTION, and MAIN PROCESS options before executing the operation from step (5) above onwards.

(12)After completing harvesting, execute the needle washing, reagent exchange (using distilled water and using air), pump drying functions as daily maintenance.



Please do drainage work of compressor once a week.
(Refer to Maintenance manual “6.3. Drainage work” for the details)

2.5 Total processing time

The processing time is as shown in the table when operating on a right condition.
The combination has "Number of ABC execution, G process execution or skip and number of IJK execution".
The Hypotonic processing time and the centrifuge time besides the above-mentioned execution time are added at the total processing time.

SET VALUE

Proc	Items	Value	Unit
A1-3	Centrifuge	300	sec
B1-3	Aspiration	19	mm
C1-3	Injection of PBS/Hypotonic	5.0	ml
C1-3	Stirring Time	5.0	sec
E	Hypotonic Stirring Time	5.0	sec
	Total Hypotonic Time	35	min
G	Injection of Pre-fixation	0.5	ml
G	Stirring Time	10	sec
H	Centrifuge	300	sec
I1	Aspiration	14	mm
J1	Injection of Fixative	5.0	ml
J1	Stirring Time	10	sec
K1	Centrifuge	300	sec
I2-5	Aspiration	9	mm
J2-5	Injection of Fixative	5.0	ml
J2-5	Stirring Time	5	sec
K2-5	Centrifuge	150	sec

Table of the total processing time

8 Samples Total Processing Time [min]			
G Execution	ABC x1	ABC x2	ABC x3
IJK = 1	56.2	56.2	56.2
IJK = 2	65.7	65.7	65.7
IJK = 3	72.6	72.6	72.6
IJK = 4	79.4	79.4	79.4
IJK = 5	86.3	86.3	86.3
G Skip	ABC x1	ABC x2	ABC x3
IJK = 1	47.2	47.2	47.2
IJK = 2	56.7	56.7	56.7
IJK = 3	63.6	63.6	63.6
IJK = 4	70.4	70.4	70.4
IJK = 5	77.3	77.3	77.3

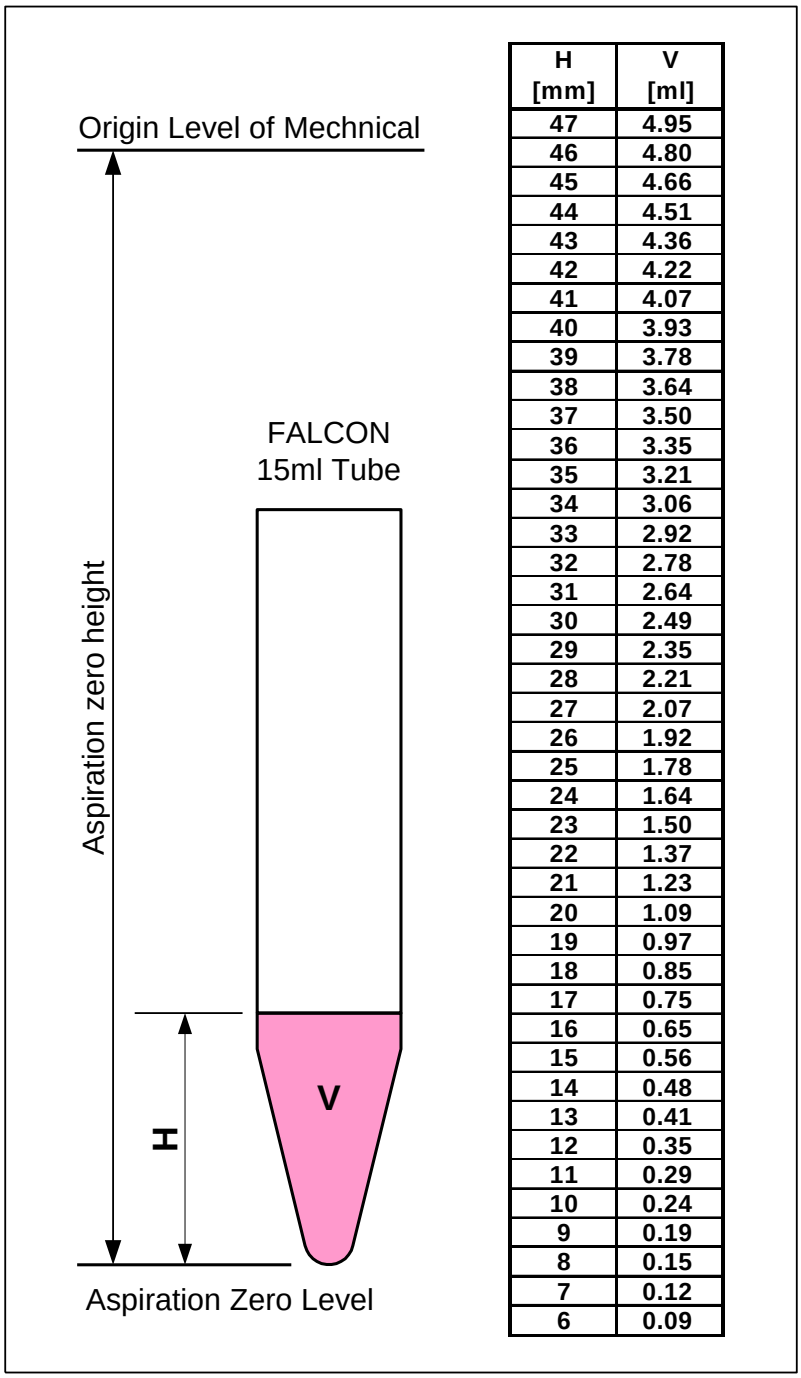
16 Samples Total Processing Time [min]			
G Execution	ABC x1	ABC x2	ABC x3
IJK = 1	62.3	62.3	62.3
IJK = 2	75.4	75.4	75.4
IJK = 3	85.7	85.7	85.7
IJK = 4	96.0	96.0	96.0
IJK = 5	106.3	106.3	106.3
G Skip	ABC x1	ABC x2	ABC x3
IJK = 1	50.6	50.6	54.6
IJK = 2	63.7	63.7	67.7
IJK = 3	74.0	74.0	78.0
IJK = 4	84.3	84.3	88.3
IJK = 5	94.6	94.6	98.6

4 Samples Total Processing Time [min]			
G Execution	ABC x1	ABC x2	ABC x3
IJK = 1	55.4	55.4	55.4
IJK = 2	64.4	64.4	64.4
IJK = 3	70.7	70.7	70.7
IJK = 4	77.1	77.1	77.1
IJK = 5	83.4	83.4	83.4
G Skip	ABC x1	ABC x2	ABC x3
IJK = 1	46.6	46.6	46.6
IJK = 2	55.6	55.6	55.6
IJK = 3	61.9	61.9	61.9
IJK = 4	68.2	68.2	68.2
IJK = 5	74.6	74.6	74.6

12 Samples Total Processing Time [min]			
G Execution	ABC x1	ABC x2	ABC x3
IJK = 1	61.6	61.6	61.6
IJK = 2	74.2	74.2	74.2
IJK = 3	83.9	83.9	83.9
IJK = 4	93.7	93.7	93.7
IJK = 5	103.4	103.4	103.4
G Skip	ABC x1	ABC x2	ABC x3
IJK = 1	50.1	50.1	52.4
IJK = 2	62.6	62.6	65.0
IJK = 3	72.4	72.4	74.7
IJK = 4	82.1	82.1	84.5
IJK = 5	91.9	91.9	94.2

(1) Setting the value of the aspiration level

The connection between the set aspiration level and remaining solution volume in the tube is as follows: You can set the aspiration level to an accuracy of 1mm with this equipment. Determine the aspiration level suitable for the actual samples for each process.



H [mm]	V [ml]
47	4.95
46	4.80
45	4.66
44	4.51
43	4.36
42	4.22
41	4.07
40	3.93
39	3.78
38	3.64
37	3.50
36	3.35
35	3.21
34	3.06
33	2.92
32	2.78
31	2.64
30	2.49
29	2.35
28	2.21
27	2.07
26	1.92
25	1.78
24	1.64
23	1.50
22	1.37
21	1.23
20	1.09
19	0.97
18	0.85
17	0.75
16	0.65
15	0.56
14	0.48
13	0.41
12	0.35
11	0.29
10	0.24
9	0.19
8	0.15
7	0.12
6	0.09

The connection between the set aspiration level and remaining solution volume in the tube

[CAUTION]
If remaining the solution in the tube after injection is set more than 7ml, it may spatter during stirring, result in serious damage to the equipment. The solution volume level in the tube should therefore be set with due caution.

(2) Aspiration level setting and reagent injectable range

If remaining the solution in the tube after injection is set more than 7.0ml, it may spatter during stirring, result in serious damage to the equipment.
Make sure to check if the relation between the setting of B and C process, I and J process are within the “OK range” in the below chart and graph.
Also, make sure to determine the setting values of B, C, and G process to not to be more than 7.0ml into the sample tube in case of selecting G process.

Aspiration level setting and range of reagent injectable volume

Aspiration level [mm]	Settable range of Hypotonic injection																			
	Settable range of fixative injection																-			
	Injection volume [ml]																			
	0.00 ~2.00	2.25	2,50	2.75	3.00	3.25	3.50	3.75	4.00	4.25	4.50	4.75	5.00	5.25	5.50	5.75	6.00	6.25	6.50	6.75	7.00 ~10.00
8																					
9																					
10																					
11																					
12																					
13																					
14																					
15																					
16																					
17																					
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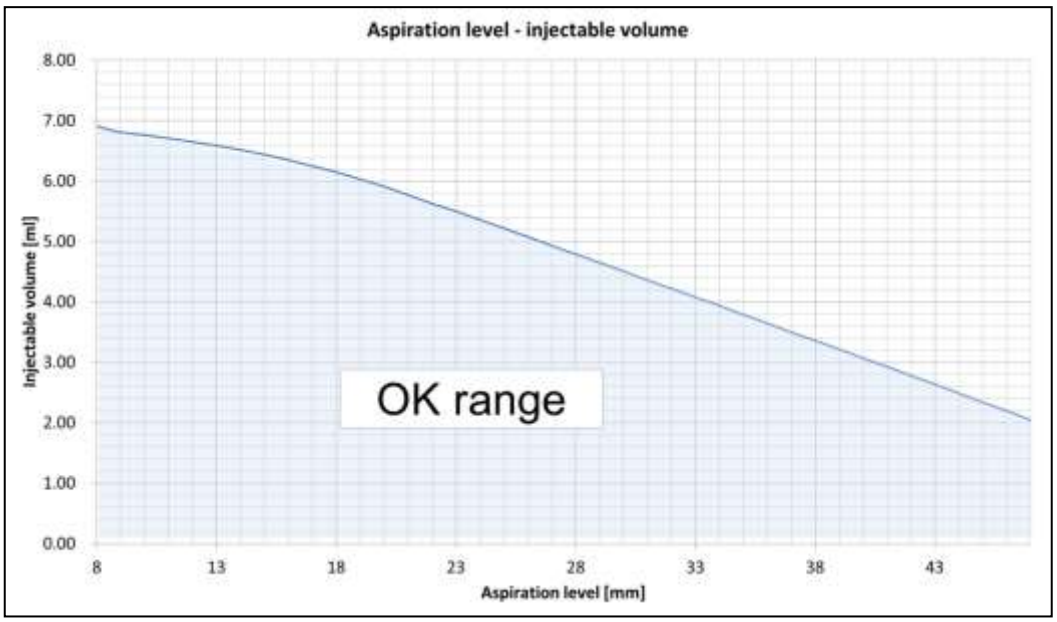
36																					
37																					
38																					
39																					
40																					
41																					
42																					
43																					
44																					
45																					
46																					
47																					

OK range

NG range



3. Explanation of the Operation Panel

This section describes how to use the operation panel of the Metaphase Harvester HANABI-PI. The description is based on the following format:

Panel Layout

Image display of the operation panel
Indication of selected key, setting range, etc.

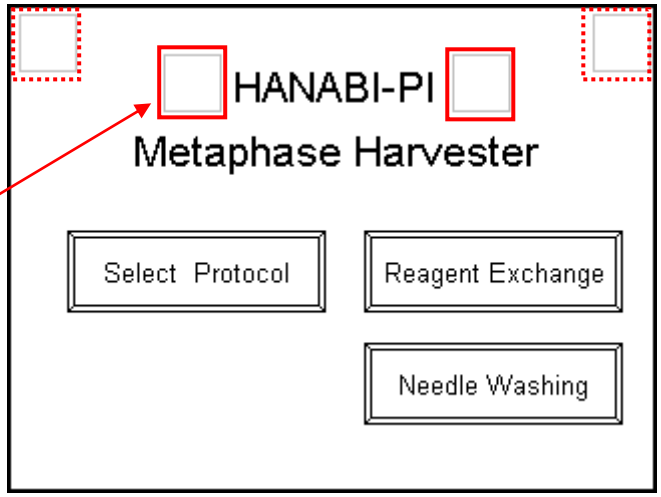
Description

(#) Operating Screen Title

< >: Indicates the name of the selected key.
[ ]: Indicates the name or description of an item.
***:** Indicates a supplementary explanation.
(Note): Indicates particularly important information to be aware of.

Sentenses to be paid attention to will be indicated in **Red** or **Blue**.

3.1. MAIN



This screen is displayed after the equipment is turned ON or the operation is completed.

<Select Protocol> key: Switches the screen to (3.4) SELECT PROTOCOL.
The user will traverse this SELECT PROTOCOL screen each time before executing the main process or setting the set items.
When <Select Protocol> is selected, the previously set protocol will be displayed.

<Reagent Exchange> key: Switches the screen to (3.2) REAGENT EXCHANGE.
The screen will revert to that used for executing the reagent exchange function.

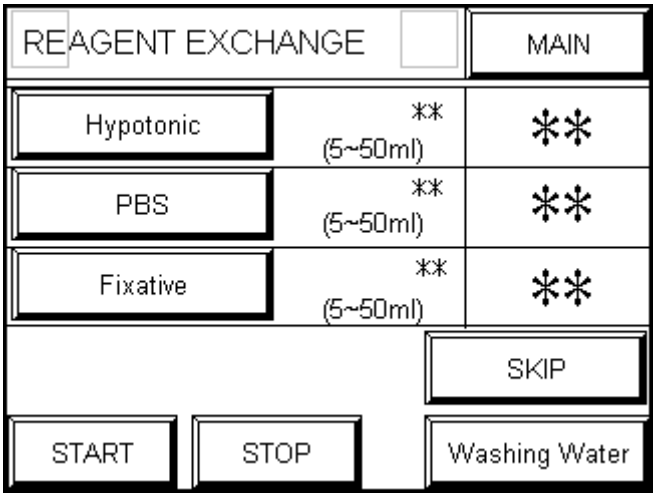
<Needle Washing> key: Switches the screen to (3.3) NEEDLE WASHING.
The screen will revert to that used for executing the Needle washing function.

<Temperature Monitor> Hidden key:
Switches the screen to (3.5) Tenperature Monitor.
When both of the two places (Screen upside both sides) are pushed within one second (When the part is pushed, Pits and the sound are heard), it moves to the Manual Operation screen. (Red broken line square part)
The temperature monitor screen can be displayed by main screen (3.1), (3.2) - (3.6), and (3.19) - (3.24).

<Manual Operation> Hidden key:
Switches the screen to Maintenance Manual (3.4) MANUAL OPERATION (TABLE).
When both of the two places (HANABI-PI both sides) are pushed within one second (When the part is pushed, Pits and the sound are heard), it moves to the Manual Operation screen. (Red solid line square part)
When the manual operation is executed, the setting of the item of the protocol under the selection content is reflected.

WARNING
<Manual Operation> Hidden key is provided only for maintenance purposes and should not be used during normal operations.

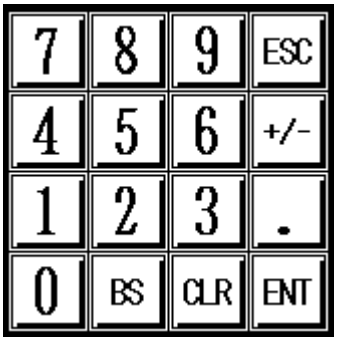
3.2. REAGENT EXCHANGE



Implement a reagent exchange before executing the main process as a routine activity. The capacity of the exchange line is 20ml for the hypotonic solution, 20ml for the PBS and 15ml for fixative solution respectively. Execute the reagent exchange function using solution volumes larger than those mentioned.

Each unit returns to the origin (Home Position) when the first start key is pushed when the origin positioning is not done.
(The interlock works. Please tighten the door for the operating.)

(1) **[Exchanging line setting]:** Specified lines are exchanged.
< Hypotonic > Key: Lines of the hypotonic solution are exchanged.
< PBS > Key: Lines of PBS are exchanged.
(When PBS is used, lines of the hypotonic solution are exchanged first of all.)
< Fixative > Key: Lines of Fixative are exchanged.
(2) **[The setting of the exchanged amounts of the reagent]**: The amount of each reagent is input. (1-50ml) It is not revokable while exchanging is starting. The amount from the start is displayed while exchanging it.
(3) **[Line refreshing setting while operating automatically]**: When injecting it, the line refreshing is executed according to the protocol during the automatic action. The amount of refreshing is set on the maintenance screen.
(4) **[Exchanging execution]**: The reagent is exchanged by the setting condition.
< Start > key: The reagent exchanging is started.
(Note) During this operation, only the STOP key is effective.
< Stop > key: Stops the operation forcibly.
a) The pump stops by the origin position after injecting solution once.
b) The needle rises and the washing water is thrown at the same time.
c) The current count number will revert back to zero.

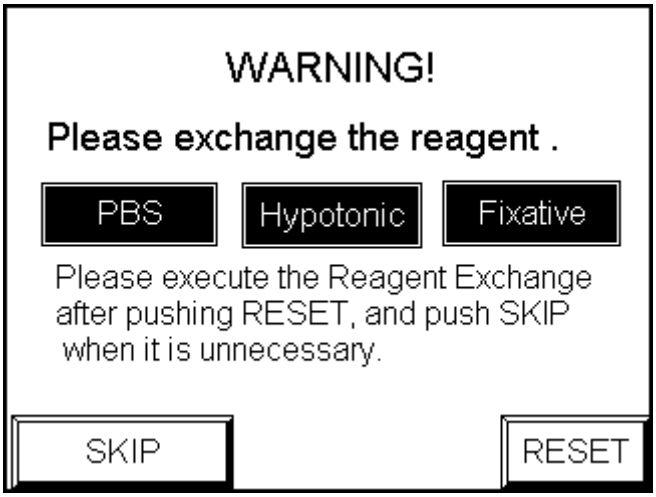


(5) **[How to change the setting values]**
Touch the setting value display, ten key is displayed.
Change the value using the numeric keypad and confirm with the ENT key. The numbers other than the range of the setting cannot be input.

< Skip > key: When the reagent need not be exchanged, it is possible to skip. Switches the screen to (3.1) MAIN.
< Washing Water > key: Washing water will be discharged in the injection part of the tray.
<MAIN> key: Switches the screen to (3.1) MAIN.

If the reagent exchanging for a set protocol is not executed, following "WARNING" is displayed.

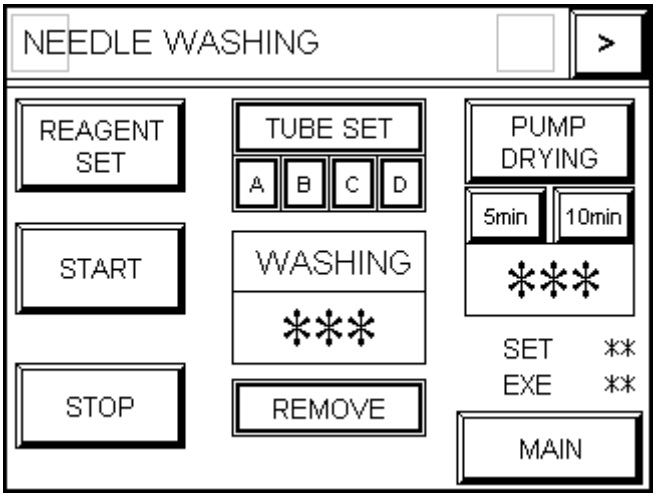
When **< SET START >** key to this process sample set screen (3.21) is pushed, the completion of exchanging the reagent is checked.



< SKIP > key: When the reagent need not be exchanged, it is possible to skip.
Switches the screen to (3.21) SET SAMPLES.
< RESET > key: Switches the screen to (3.21) SET SAMPLES.

Please execute exchanging the reagent (PBS, Hypotonic, and Fixative) according to this protocol when last time and this protocol are different.
Please change the item setting after exchanging the reagent.
(The protocol change can be previously executed.)

3.3. NEEDLE WASHING



Procedure for needle washing
Wash the needle with washing water as part of the aspiration needle daily maintenance.
(The interlock works. Please tighten the door for the operating.)

(1) **[Setting]**
<REAGENT SET> key: **Remains lit up during operation.**

a) Station #1 will be positioned at the setting position.
b) When the positioning process is completed, the positioning completion lamp will light up and the buzzer will sound.
c) Open the cover and set 4 (position A, B, C and D) tubes with washing water, before closing the cover securely.
d) When the sensor confirms that the tubes are set, the **<START>** key will blink.

<STOP> key:

a) When the key is pressed while the table is moving, St. #1 will be positioned at the setting position and the position will then descend.
b) When the key is pressed during positioning, the position will descend and return to the original point.
c) Once the positioning is complete, the key will be disabled.

(2) **[Washing]**
<START> key: **Effective only when the key is blinking.**
(The key remains lit up during operation.)

a) Station #1 will be positioned at the aspiration position.
b) The aspiration needle will move to the tube A, B, C and D before descending to the 0.2ml position and remaining there for 3 minutes. (This operation is carried out to wash the outer needle wall.)
c) Once the washing water in the tube is vacuumed up, the aspiration needle will ascend to return to the moving origin (washing position). It will then descend to the 0.2ml position and stop there.
d) The washing water will be discharged and all vacuumed up.
e) After repeating the washing operation (above d) three times, the needle will ascend. (This operation is carried out to wash the inner needle wall.)
f) Once the operation is complete, the **< REMOVE >** will blink. Station #1 will return to setting position automatically.

<STOP> key:

a) Returns the aspiration needle to the moving original point.
b) When the key is pressed while the table is moving, St. #1 will be positioned at the setting position and the position will then descend.

(3) **[Removing]**

< REMOVE > key: **Effective only when the key is blinking.**
(The key remains lit up during operation.)

a) Remove the four empty tubes.
b) When the sensor confirms the removal of the four tubes, the **<STOP>** key will blink.

<STOP> key:

a) The operation will stop and the tube will return to the origin. The position will then descend.
b) When the key is pressed while the table is moving, St. #1 will be positioned at the setting position and the position will then descend.

(4) **Procedure for pump drying**

(This operation is executed following the needle washing automatically.)
This operation is carried out to dry the inside of the aspiration pump following execution of the main process.
The previously set value will be highlighted.

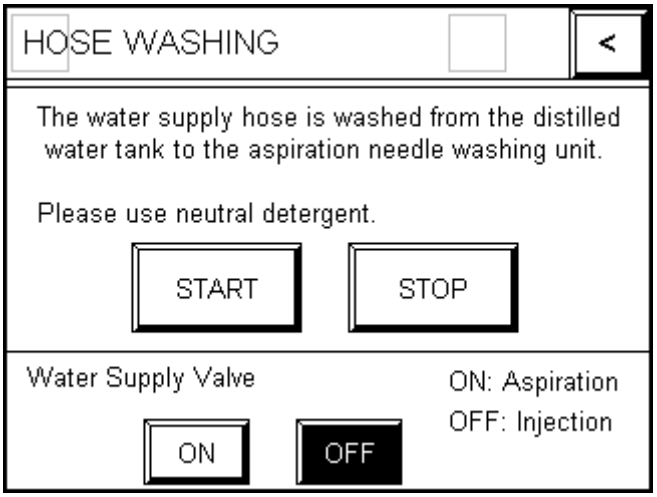
<5min> key: The drying time will be set to 5 minutes.
<10min> key: The drying time will be set to 10 minutes.
<PUMP DRY> key: The aspiration pump in the aspiration section will be turned ON, and then turned OFF 5 (or 10) minutes) later. Restarting is acceptable.
<STOP> key: Stops the operation forcibly.
The aspiration pump in the aspiration part will be turned OFF.

(5) **[How to change the setting values]**

Touch the setting value display, ten key is displayed.
Change the value using the numeric keypad and confirm with the ENT key. The numbers other than the range of the setting cannot be input.

<MAIN> key: Switches the screen to (3.1) MAIN.
< ▶ > key: Switches the screen to (3.4) HOSE WASHING.

3.4. HOSE WASHING



The water supply hose from the distilled water tank to the aspiration needle washing part is washed. The water supply valve is switched. (For water purging in water supply line)

Please connect the hose connected with the distilled water tank with the container that the cleaning solution (surface-active agent liquid such as soapless soaps) entered.

< START > key: The water supply and the aspiration pump are operated at the same time.

<STOP> key: Stops the operation forcibly.
Stop it when the cleaning solution container empties.
The water supply and the aspiration pump are turned off.

Please change the hose connection into the distilled water tank after washing the hose.

< ◀ **>** key: Switches the screen to (3.3) NEEDLE WASHING.

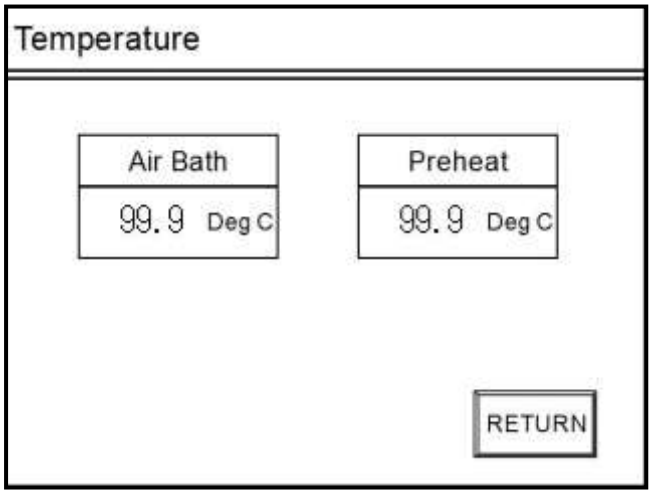
Water supply switch valve:
The water purging in piping is done with the valve that switches flow from the distilled water tank to the aspiration needle washing part or the reagent flash part washing.

< ON > key: The valve is switched to drain (aspiration needle washing part) side.

< OFF > key: The valve is switched to injection (reagent flash tray) side.

[CAUTION]
When the check valve freezes by keeping or the movement, the check valve is damaged. Do not make it to the low temperature while keeping and moving the instrument. Execute the water purging of the check valve.

3.5. Temperature Monitor

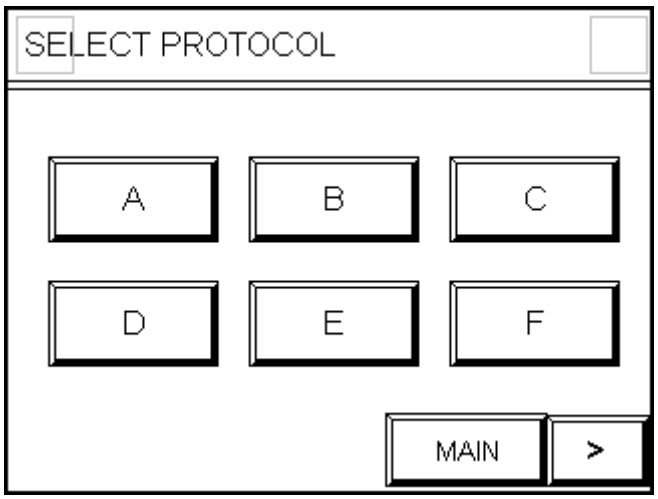


The temperature of Air Bath and the Preheat (PBS/hypotonic solution) is displayed.
The control temperature of Air Bath and the Preheat can be read.

This screen is displayed to push both upside both sides of the screen (3.1) - (3.7) and (3.20) - (3.24) with moving making and the hidden key from **<Temperature Monitor> Hidden key** within one second (When the part is pushed, Pits and the sound are heard).

<Return> key: Return to previous screen.

3.6. SELECT PROTOCOL



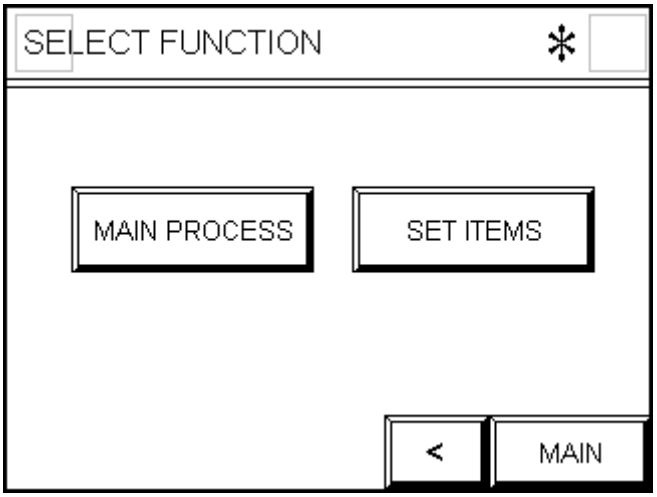
The screen is switched from the MAIN setting before the main process is executed.
The previously set value will be highlighted.

The protocol executed in the previous operation will be highlighted. 6 different protocols, A, B, C, D, E and F, can be set and registered.

< A > key: The setting A will be selected.
The main process will be executed according to the setting A.
< B > key: The setting B will be selected.
The main process will be executed according to the setting B.
< C > key: The setting C will be selected.
The main process will be executed according to the setting C.
< D > key: The setting A will be selected.
The main process will be executed according to the setting D.
< E > key: The setting B will be selected.
The main process will be executed according to the setting E.
< F > key: The setting C will be selected.
The main process will be executed according to the setting F.

<MAIN> key: Switches the screen to (3.1) MAIN.
< ▶ **>** key: Switches the screen to (3.7) SELECT FUNCTION.

3.7. SELECT FUNCTION



The currently selected protocol will be displayed in the top right corner of the screen until the main process is completed.

The screen is switched from SELECT PROTOCOL to enable items to be set for the main process and the main process to then be executed.

<MAIN PROCESS> key: Switches the screen to (3.20) SET SAMPLES NUMER.
The main process will then be executed.

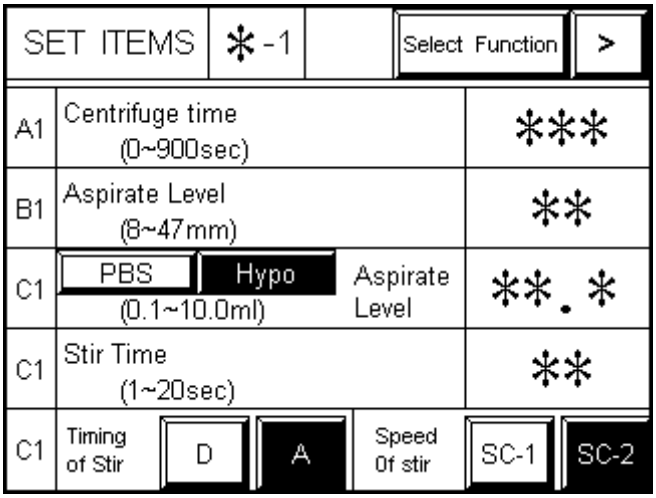
The setting contents of the currently selected protocol will be confirmed.

<SET ITEMS> key: Switches the screen to (3.8) SET ITEMS at MAIN.
Set the items of each process for the selected protocol.

< ◀ **>** key: Switches the screen to (3.6) SELECT PROTOCOL.

<MAIN> key: Switches the screen to (3.1) MAIN.

3.8. SET ITEMS at MAIN (Processes A1, B1, and C1)



First ABC process setting: Set the centrifuging time for the A-process, aspiration level (B) for the B-process, PBS/hypotonic solution injection volume (C) and stirring time/timing/speed for the C-process.

(1) **[Centrifuging time]**

The setting time includes the rise time (about 18 seconds), but not the fall time (warm-down time, about 25 seconds).
The Air Bath control is turned off while centrifuging.
The centrifuge time is set within the range of (0-900sec).
Setting 0 can skip the centrifuge.

V [ml]	H [mm]
0.15	8
0.2	9
0.3	11
0.5	14
1.0	19
1.5	23
2.0	27
2.5	30
3.0	34
3.5	37
4.0	40
4.5	44
5.0	47

(2) **[Aspiration level]**: Displayed in height terms.
Setting range: 47mm = 5.0ml level
8mm = 0.15ml level

(3) **[PBS/Hypotonic solution injection volume]**
The value can be set at intervals of 0.1ml.

Set the value such that the total volume of the solution volume remaining in the tube at the aspiration level (B), PBS/Hypotonic solution injection volume (C), and Fixative solution injection volume (G) in G-process becomes 7.0ml or less.

Example: B=1.0ml, C=5.5ml, G=0.5ml TOTAL: 7.0ml

The amount of the injection of the PBS/Hypotonic solution can set even 10ml or less. PBS/Hypotonic is injected, divided into two portions. The injection and the stir of 5ml are done in the beginning. Next, it injects it without stirring the hypotonic solution for the remainder of a set amount.

Please select G process skip (G-Skip) at the setting that exceeds 6ml.

[CAUTION]
If remaining the solution in the tube after injection is set more than 7.0ml, it may spatter during stirring, result in serious damage to the equipment. The solution volume level in the tube should therefore be set with due caution.

(4) **[Stirring]**

The stirring rate increases in two stages the rotation speed is a half of the set speed for the first 1 second.

The stir timing can be set to two ways. These are <D>key (Stir during injection) and <A>key (Stir after injection). The stir speed can be set to two stages <SC-1>,< SC-2>key). The stir speed setting opens and adjusts a front right door. (Refer to the photograph.)

The rotational speed and the scale in the stir part are about as follows.

Factory Setting

SC-1 = 1500rpm
SC-2 = 2100rpm(Max)

(%) - Speed (rpm)
0-100rpm
10-420rpm
20-730rpm
30-1030rpm
40-1320rpm
50-1610rpm
60-1910rpm
Max-2100rpm

(5) **[How to change the setting values]**

Touch the setting value display, ten key is displayed.

Change the value using the numeric keypad and confirm with the ENT key. The numbers other than the range of the setting cannot be input.

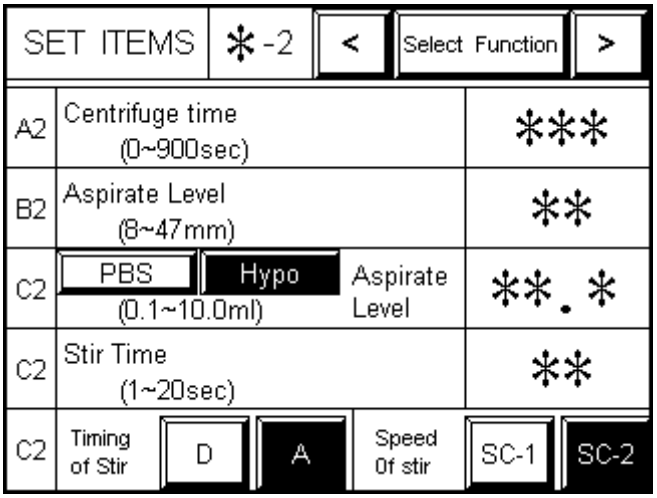
<SELECT FUNCTION> key: Switches the screen to (3.7) SELECT FUNCTION.

< ▶ **>** key: Switches the screen to (3.9) SET ITEMS at MAIN (Processes A2, B2, and C2).

*** The previously set values are stored for each setting value and displayed when each setting screen appears.**

*** The values in parentheses indicate the adjustable range. Any value outside this range is unacceptable.**

3.9. SET ITEMS at MAIN (Processes A2, B2, and C2)



Second ABC process setting: Set the centrifuging time for the A-process, aspiration level (B) for the B-process, PBS/hypotonic solution injection volume (C) and stirring time/timing/speed for the C-process.

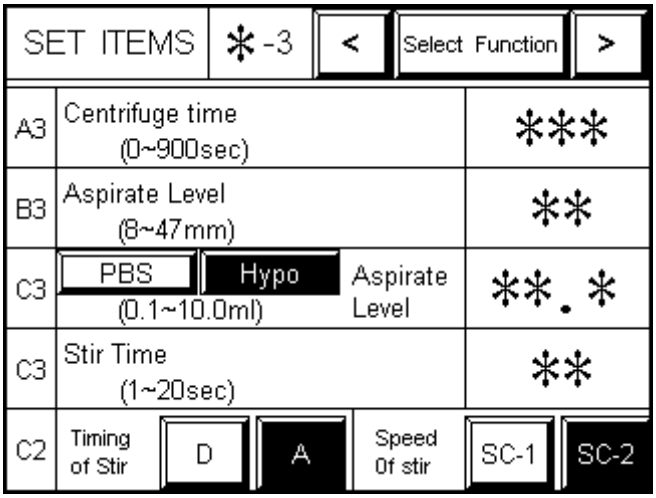
The setting method is the same as (3.8).

(1) **[How to change the setting values]**

Touch the setting value display, ten key is displayed.
Change the value using the numeric keypad and confirm with the ENT key. The numbers other than the range of the setting cannot be input.

<SELECT FUNCTION> key: Switches the screen to (3.7) SELECT FUNCTION.
< ▶ **>** key: Switches the screen to (3.10) SET ITEMS at MAIN (Processes A2, B2 and C2).
< ◀ **>** key: Switches the screen to (3.8) SET ITEMS at MAIN (Processes A1, B1, and C1).

3.10. SET ITEMS at MAIN (Processes A3, B3, and C3)



Third ABC process setting: Set the centrifuging time for the A-process, aspiration level (B) for the B-process, PBS/hypotonic solution injection volume (C) and stirring time/timing/speed for the C-process.

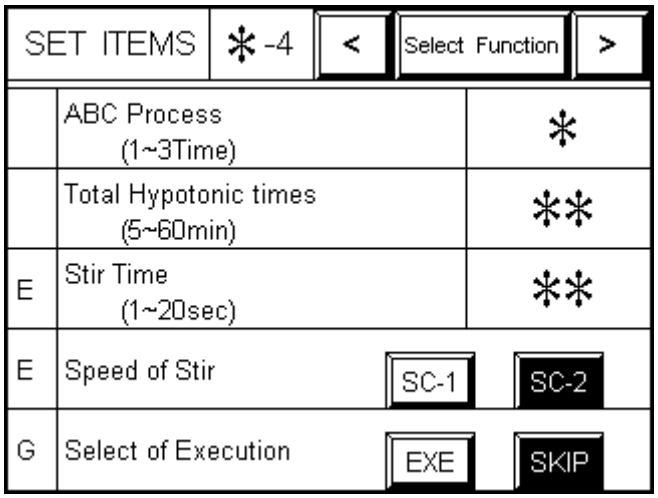
The setting method is the same as (3.8).

(1) **[How to change the setting values]**

Touch the setting value display, ten key is displayed.
Change the value using the numeric keypad and confirm with the ENT key. The numbers other than the range of the setting cannot be input.

<SELECT FUNCTION> key: Switches the screen to (3.7) SELECT FUNCTION.
< ▶ **>** key: Switches the screen to (3.11) SET ITEMS at MAIN (Processes E and G).
< ◀ **>** key: Switches the screen to (3.9) SET ITEMS at MAIN (Processes A2, B2, and C2).

3.11. SET ITEMS at MAIN (Processes E and G)



Set the ABC process count, total hypotonic time, stirring time/timing/speed for the E-process for the hypotonic rest and select of the G-process execute/skip.

(1) **[ABC process count]**
Tow counts = A, B, and C processes will be executed twice.
The maximum, set value is three.

(2) **[Total hypotonic time]**
Enter the total hypotonic time between 5 to 60 minutes. Total hypotonic time is setting 20 minutes or more. Total hypotonic processing time is defined from hypotonic solution injection (C2 when PBS is injected with C1) at the time of the first fixative solution injection (G or J1). Therefore, the rest time changes by the number of ABC execution and G process skip.

* **[Hypotonic Rest Time]**
Please refer to the calculation type at the Rest time of the hypotonic solution for (3.22) Main process progress (A,B,C,D) for the Rest time.
However, the Rest time of D and F becomes 0 seconds at the following setting.
(a) ABC=1 & C1=PBS
(b) ABC=2 & C1=C2=PBS
(c) ABC=3 & C1=C2=C3=PBS

(3) **[Stirring Time]** Setting of E process stir time

(4) **[Stir Speed]** The stir speed can be set to two stages (SC-1, SC-2).

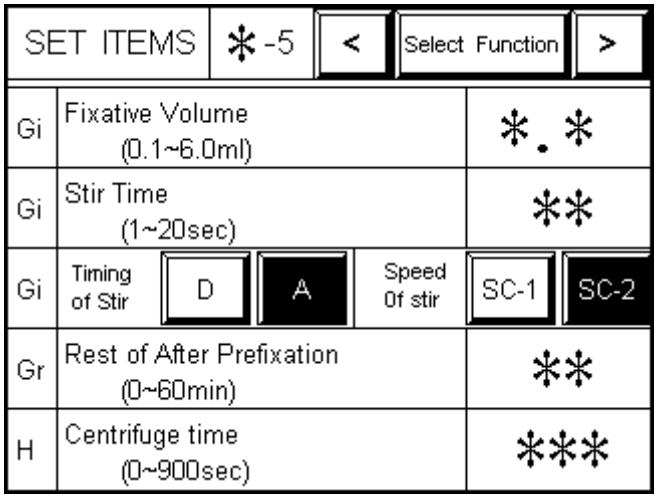
(5) **[G Select of Execution]**
< EXE > key: The G-process will be executed.
< SKIP > key: The G-process will not be executed.

When G process is skipped, fixative solution will be injected for the first time in the process (J1) through centrifuge (H) and process (I1).

(6) **[How to change the setting values]**
Touch the setting value display, ten key is displayed.
Change the value using the numeric keypad and confirm with the ENT key. The numbers other than the range of the setting cannot be input.

<SELECT FUNCTION> key: Switches the screen to (3.7) SELECT FUNCTION.
< ▶ **>** key: Switches the screen to (3.12) SET ITEMS at MAIN (Process G and H).
< ◀ **>** key: Switches the screen to (3.10) SET ITEMS at MAIN (Processes A3, B3, and C3).

3.12. SET ITEMS at MAIN (Processes G and H)



Set injection volume of the first Fixative solution, stirring time/timing/speed for the G-process, rest time of the first Fixative, and centrifuge time for the H-process.

(1) **[Fixative solution injection volume (G)]**

The value can be set at intervals of 0.1ml and is automatically converted:

Set the value such that the total volume of the solution volume remaining in the tube at the aspiration level (B), Hypotonic solution injection volume (C), and Fixative solution injection volume (G) in G-process becomes 7.0ml or less.

Example: B=1.0ml, C=5.5ml, G=0.5ml TOTAL: 7.0ml

[CAUTION]
If remaining the solution in the tube after injection is set more than 7.0ml, it may spatter during stirring, result in serious damage to the equipment. The solution volume level in the tube should therefore be set with due caution.

(2) **[Stirring time] for G-process**

The stir timing can be set to two ways. These are <D>key (Stir during injection) and <A>key (Stir after injection). The stir speed can be set to two stages <SC-1>,< SC-2>key.

(3) **[Rest time] for G-process**

Rest time can set it from 0 to 60 minutes. The zero out doesn't execute rest time.

(4) **[Centrifuging time]**

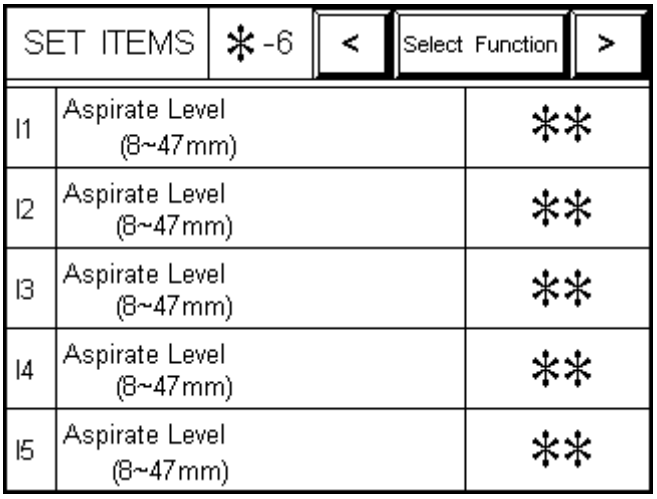
The setting time includes the rise time (about 18 seconds), but not the fall time (warm-down time, about 25 seconds).

(5) **[How to change the setting values]**

Touch the setting value display, ten key is displayed.
Change the value using the numeric keypad and confirm with the ENT key. The numbers other than the range of the setting cannot be input.

<SELECT FUNCTION> key: Switches the screen to (3.7) SELECT FUNCTION.
< ▶ **>** key: Switches the screen to (3.13) SET ITEMS at MAIN (Process I).
< ◀ **>** key: Switches the screen to (3.11) SET ITEMS at MAIN (Processes E and G).

3.13. SET ITEMS at MAIN (Process I)



Set the aspiration levels I1, I2, I3, I4 and I5 for the I-process.

(1) **[Aspiration level]:**

A point inside in the sample tube is zero levels. The setting is units of mm in the direction of height.

It is not height from a mechanical starting point of the needle for suction.

Setting conditions for the I-process aspiration level (I1):
When the J-process Fixative solution injection volume (J1)
is 5.0ml: Shall be set to 27 or lower.
For 2.0ml or less: No limitation in setting value.

V [ml]	H [mm]
0.15	8
0.2	9
0.3	11
0.5	14
1.0	19
1.5	23
2.0	27
2.5	30
3.0	34
3.5	37
4.0	40
4.5	44
5.0	47

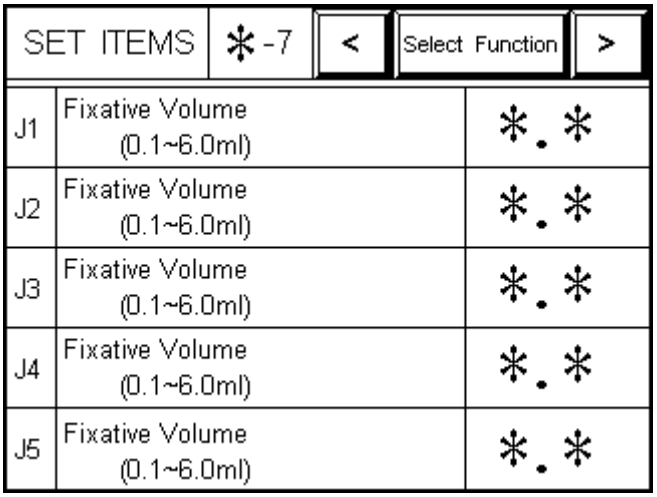
[CAUTION]
If remaining the solution in the tube after injection is set more than 7.0ml, it may spatter during stirring, result in serious damage to the equipment. The solution volume level in the tube should therefore be set with due caution.

(2) **[How to change the setting values]**

Touch the setting value display, ten key is displayed.
Change the value using the numeric keypad and confirm with the ENT key. The numbers other than the range of the setting cannot be input.

<SELECT FUNCTION> key: Switches the screen to (3.7) SELECT FUNCTION.
< ▶ **>** key: Switches the screen to (3.14) SET ITEMS at MAIN (Process J).
< ◀ **>** key: Switches the screen to (3.12) SET ITEMS at MAIN (Processes G and H).

3.14. SET ITEMS at MAIN (Process J)



Set the fixative solution injection volumes (J1, J2, J3, J4 and J5) for the J-process.

(1) **[Fixative solution injection volume]**

The value can be set at intervals of 0.1ml.

J-process Fixative solution injection volume (J1):

When the I-process aspiration level (I1) is 40mm: Shall be set to 2.0ml or lower.

For the level (I1) of 19mm or lower: No limitation in setting value.

[CAUTION]

If remaining the solution in the tube after injection is set more than 7.0ml, it may spatter during stirring, result in serious damage to the equipment. The solution volume level in the tube should therefore be set with due caution.

(2) **[How to change the setting values]**

Touch the setting value display, ten key is displayed.

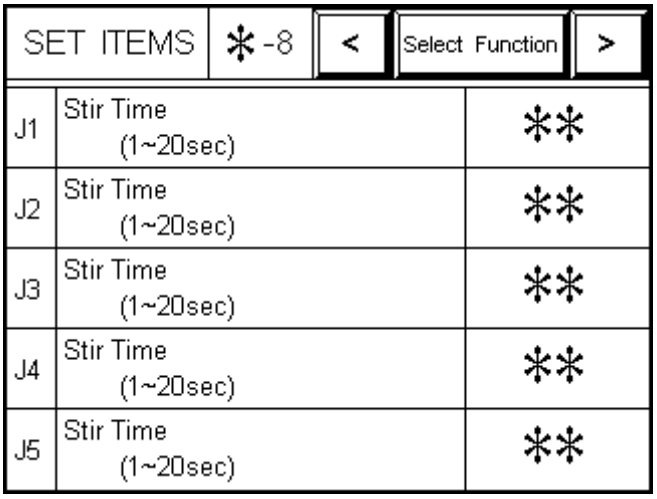
Change the value using the numeric keypad and confirm with the ENT key. The numbers other than the range of the setting cannot be input.

<SELECT FUNCTION> key: Switches the screen to (3.7) SELECT FUNCTION.

< ▶ **>** key: Switches the screen to (3.15) SET ITEMS at MAIN (Processes J).

< ◀ **>** key: Switches the screen to (3.13) SET ITEMS at MAIN (Process I).

3.15. SET ITEMS at MAIN (Process J)



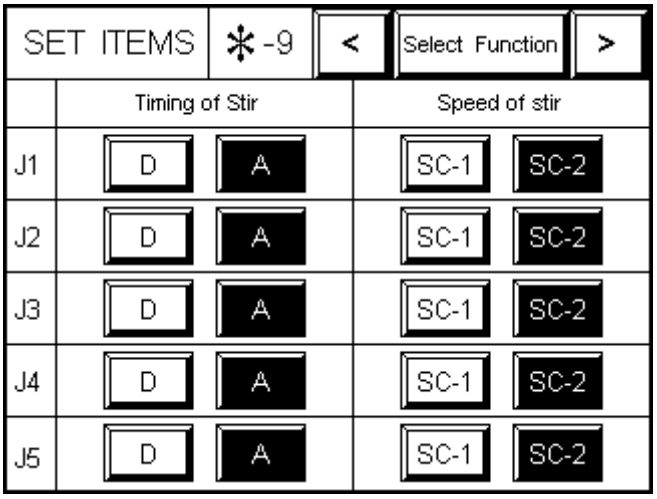
Set the Fixative solution stirring time (J1, J2, J3, J4 and J5) for the J-process.

(1) **[Fixative solution stirring time]**
The stir time can be set respectively of J1-J5.

(2) **[How to change the setting values]**
Touch the setting value display, ten key is displayed.
Change the value using the numeric keypad and confirm with the ENT key. The numbers other than the range of the setting cannot be input.

<SELECT FUNCTION> key: Switches the screen to (3.7) SELECT FUNCTION.
< ▶ **>** key: Switches the screen to (3.16) SET ITEMS at MAIN (Processes J).
< ◀ **>** key: Switches the screen to (3.14) SET ITEMS at MAIN (Process I).

3.16. SET ITEMS at MAIN (Process J)



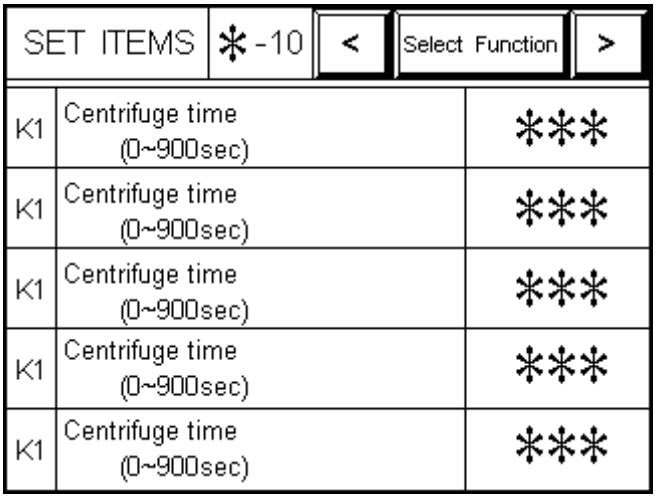
Set the fixative solution stirring timing and speed (J1, J2, J3, J4 and J5) for the J-process.

(1) **[Fixative solution stirring timing]**
The stir timing can be set to two ways D (During) and A (After).

(2) **[Fixative solution stirring speed]**
The stir speed can be set to two stages (SC-1, SC-2).

<SELECT FUNCTION> key: Switches the screen to (3.7) SELECT FUNCTION.
< ▶ **>** key: Switches the screen to (3.17) SET ITEMS at MAIN (Processes K).
< ◀ **>** key: Switches the screen to (3.15) SET ITEMS at MAIN (Process J).

3.17. SET ITEMS at MAIN (Process K)



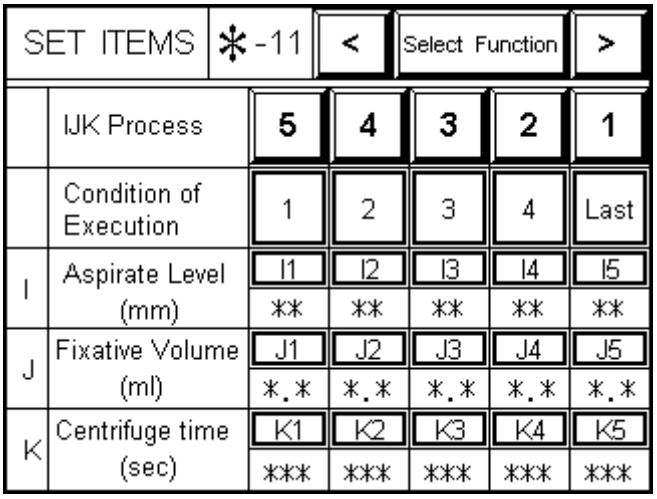
Set the fixative solution centrifuge time (K1, K2, K3, K4 and K5) for the K-process.

(1) **[Centrifuging time]**
The setting time includes the rise time (about 18 seconds), but not the fall time (warm-down time, about 25 seconds.)

(2) **[How to change the setting values]**
Touch the setting value display, ten key is displayed.
Change the value using the numeric keypad and confirm with the ENT key. The numbers other than the range of the setting cannot be input.

<SELECT FUNCTION> key: Switches the screen to (3.7) SELECT FUNCTION.
< ▶ **>** key: Switches the screen to (3.18) SET ITEMS at MAIN (Processes I,J,K).
< ◀ **>** key: Switches the screen to (3.16) SET ITEMS at MAIN (Process J).

3.18. SET ITEMS at MAIN (Processes I, J and K)



Set the IJK process count.
The connection between the set process count and operation contents is as follows:

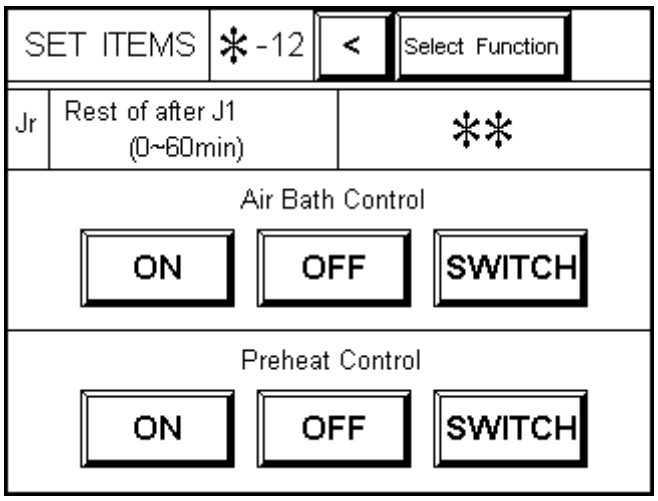
(1) **[IJK process count setting]**
< 5 > key: Executes 1 (IJK), 2 (IJK), 3 ((IJK), 4 ((IJK), and Last ((IJK).
< 4 > key: Executes 1 (IJK), 2 (IJK), 3 ((IJK), and Last ((IJK).
< 3 > key: Executes 1 (IJK), 2 (IJK), and Last ((IJK).
< 2 > key: Executes 1 (IJK) and Last ((IJK).
< 1 > key: Executes Last ((IJK).

When a process count is pressed, the corresponding process contents will be highlighted.

(2) **[How to change the setting values]**
Touch the setting value display, ten key is displayed.
Change the value using the numeric keypad and confirm with the ENT key. The numbers other than the range of the setting cannot be input.

<SELECT FUNCTION> key: Switches the screen to (3.7) SELECT FUNCTION.
< ▶ **>** key: Switches the screen to (3.19) SET ITEMS at MAIN (Processes Jr).
< ◀ **>** key: Switches the screen to (3.17) SET ITEMS at MAIN (Processes K).

3.19. SET ITEMS at MAIN (Processes Jr)



Set the Jr Rest time of after J1, Air Bath control mode and preheat control mode.

(1) **[Rest time] for after the J1process**
Rest time can set it from 0 to 60 minutes. The zero out doesn't execute rest time.
After injecting J5, Jr becomes effective at setting (IJK = 1).

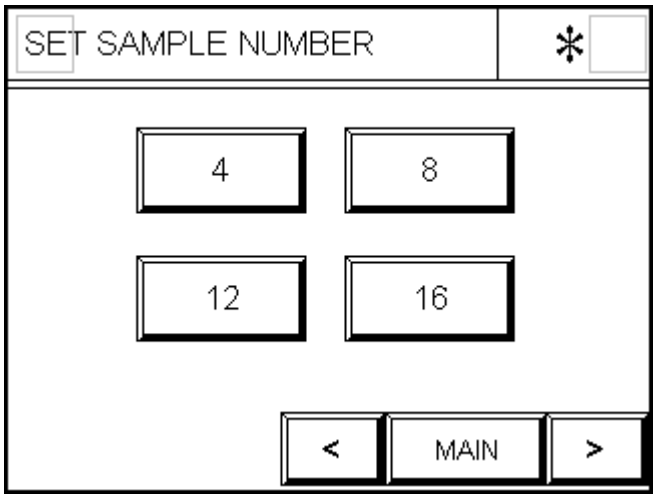
(2) **[Air bath Control mode]**
< ON > key: The temperature is continuously controlled while the power is on.
< OFF > key: The air bath temperature control is turned OFF.
<SWITCH> key: The temperature control is turned off from the G-process due to the sample removal.

(3) **[Preheat Control mode]**
< ON > key: The temperature is continuously controlled while the power is on.
< OFF > key: The preheat temperature control is turned OFF.
<SWITCH> key: The temperature control is turned off from the G-process due to the sample removal.

(4) **[How to change the setting values]**
Touch the setting value display, ten key is displayed.
Change the value using the numeric keypad and confirm with the ENT key. The numbers other than the range of the setting cannot be input.

<SELECT FUNCTION> key: Switches the screen to (3.7) SELECT FUNCTION.
< ◀ **>** key: Switches the screen to (3.18) SET ITEMS at MAIN (Processes I,J,K).

3.20. SET SAMPLES NUMBER



Set the number of samples to be processed.
The previously set contents will be highlighted when the screen appears.

(1) **[Set samples number]**
Touch the corresponding display key to change the setting.
(The selected display key will be highlighted.)

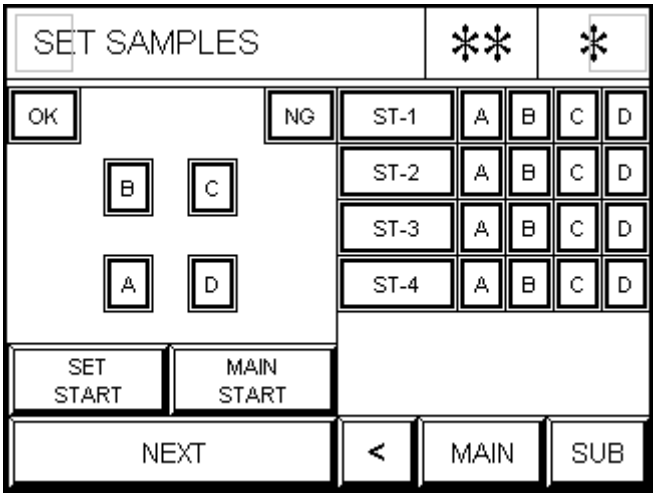
< 4 > key: Set sample tubes at Stations #1(AC) and #3(AC).
< 8 > key: Set sample tubes at Stations #1(ABCD), #3(ABCD)
< 12 > key: Set sample tubes at Stations #1(ABCD), #2(AC), #3(ABCD), #4(AC)
< 16 > key: Set sample tubes (ABCD) of the Stations #1, #2, #3 and #4.

When the number of samples doesn't come up, the dummy tube is inserted.
Refer to “Combination of samples and dummy samples (4.3)”.

<MAIN> key: Switches the screen to (3.1) MAIN.

< ◀ **>** key: Switches the screen to (3.6) SELECT PROTOCOL.
< ▶ **>** key: Switches the screen to (3.21) SET SAMPLES.

3.21. SET SAMPLES



This is a screen for setting sample tubes to the stations.
Check all the 4 stations.
Stations A, B, C and D that correspond to the set sample number will be highlighted when the screen appears.

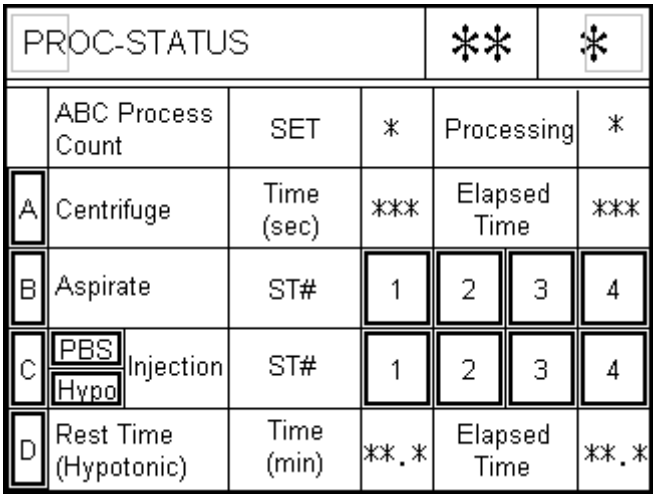
(1) **[Procedures]**

a) **<SET START>** key: Station #1 will then be positioned at the setting position.
b) When the positioning is completed, the positioning completion lamp will light up and the buzzer will sound.
Do not touch the stations before the lamp lights up and the buzzer sounds.
If you do so, it may result in a positioning failure.
c) Open the station lid.
Be sure to set tubes, and then close the lid securely.
If the lid is not closed securely, samples will not be sufficiently stirred,
which may result in harvesting failure.
The sensor checks that the number of samples set corresponds to the number of samples selected.
-> When they match, [**OK**] will be highlighted.
-> If not, [**NG**] will be highlighted.
If [**NG**] is highlighted, remove the tube that has been set so that [**OK**] will be highlighted.
d) When [**OK**] is highlighted, the **<NEXT>** key will blink.
e) **<NEXT>** key: The next station will be positioned.
f) When all four stations are checked, the **<MAIN START>** key will blink.
g) Close the door of the centrifuge and main door.
h) **<MAIN START>** key: Press and hold the key for 3 seconds to start operation.
If the instruction is accepted, the buzzer will sound and the screen will change.

<MAIN> key: Switches the screen to (3.1) MAIN.
< SUB > key: Switches the screen to (3.27) SUB Press and hold the appropriate key to select the setting. This operation allows the user to respond to special protocols, such as starting operation from the G-process. Selecting SUB will trigger the same status as that when MAIN is executed during the main process (such as STOP, etc.).

< ◄ **>** key: Switches the screen to (3.20) SET SAMPLES NUMBER.
If this key is pressed during setting, the set information will be cleared.

3.22. PROCEDURE STATUS (Processes A, B, C, and D)



The progress in the main process will be displayed.

The name of the process (A, B, C, or D) and the station being executed will blink.
Once the process is complete, the display of the corresponding process will be highlighted.

The centrifuging and hypotonic times will be indicated in the count-up display from the start time.

The ABC Count Number indicates the number of the process during the execution and setting value functions.

Once the process D Hypotonic Rest Time is complete, the screen will switch to (3.23) PROCEDURE STATUS (Processes E, F, G, and H).

Processing Time Table

No.	Process	Process Items	Process Time [sec]		Setting Values
			8	16	
1	A1	Centrifuge	333	333	Centrifuge time (5min)
2	B1	Aspiration	67	131	
3	C1	Injection of Hypotonic	162	304	Injection (5ml), Stirring time (10sec)
4	A2	Centrifuge	333	333	Centrifuge time (5min)
5	B2	Aspiration	67	131	
6	C2	Injection of Hypotonic	162	304	Injection (5ml), Stirring time (10sec)
7	A3	Centrifuge	333	333	Centrifuge time (5min)
8	B3	Aspiration	67	131	
9	C3	Injection of Hypotonic	162	304	Injection (5ml), Stirring time (10sec)
10	G	Injection of First Fixative	119	218	Injection (5ml), Stirring time (10sec)
11	H	Centrifuge	333	333	Centrifuge time (5min)
12	I1	Aspiration	67	131	
13	J1	Injection of Fixative	172	324	Injection (5ml), Stirring time (10sec)

Calculation at time of process D and F

The ABC number of times, the number of samples, and the Rest time by the combination of Total Hyponic Time are calculated from the Processing Time Table in each process.
The Processing Time Table is shown above.

(1) **Rest time (D+F) is calculated by the next expression.**

Rest time (D+F) = total Hypotonic processing time- processing execution time

The processing execution time is the following processes.

(ABCx1, G-Exe.) = C1+E
(ABCx2, G-Exe.) = C1+A2+B2+C2+E
(ABCx3, G-Exe.) = C1+A2+B2+C2+A3+B3+C3+E
(ABCx1, G-Skip) = C1+E+H+I1
(ABCx2, G-Skip) = C1+A2+B2+C2+E+H+I1
(ABCx3, G-Skip) = C1+A2+B2+C2+A3+B3+C3+E+H+I1

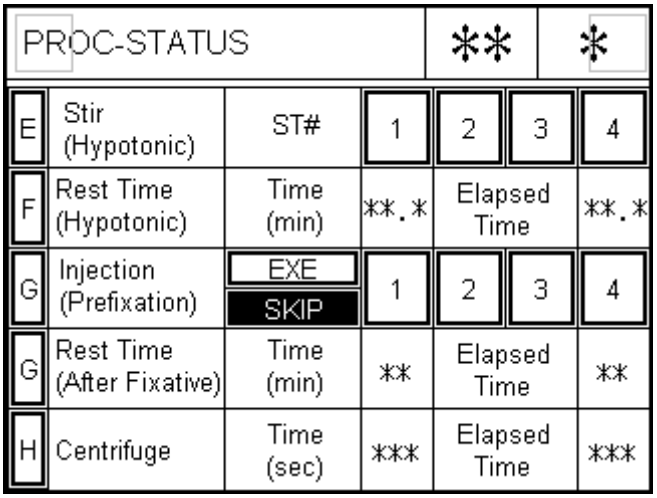
Calculation at Hypotonic Rest Time

Total Hypotonic	ABC	G Process Execution [sec]				G Process Skip [sec]			
		4	8	12	16	4	8	12	16
20	1	1,071	1,038	929	896	671	638	465	432
	2	542	476	194	128	142	76	-270	-336
	3	13	-86	-541	-640	-388	-487	-1,005	-1,104
25	1	1,371	1,338	1,229	1,196	971	938	765	732
	2	842	776	494	428	442	376	30	-36
	3	313	214	-241	-340	-88	-187	-705	-804
30	1	1,671	1,638	1,529	1,496	1,271	1,238	1,065	1,032
	2	1,142	1,076	794	728	742	676	330	264
	3	613	514	59	-40	212	113	-405	-504
35	1	1,971	1,938	1,829	1,796	1,571	1,538	1,365	1,332
	2	1,442	1,376	1,094	1,028	1,042	976	630	564
	3	913	814	359	260	512	413	-105	-204
40	1	2,271	2,238	2,129	2,096	1,871	1,838	1,665	1,632
	2	1,742	1,676	1,394	1,328	1,342	1,276	930	864
	3	1,213	1,114	659	560	812	713	195	96
45	1	2,571	2,538	2,429	2,396	2,171	2,138	1,965	1,932
	2	2,042	1,976	1,694	1,628	1,642	1,576	1,230	1,164
	3	1,513	1,414	959	860	1,112	1,013	495	396
Processing time of Hypotonic	1	129	162	271	304	529	562	735	768
	2	658	724	1,006	1,072	1,058	1,124	1,470	1,536
	3	1,187	1,286	1,741	1,840	1,588	1,687	2,205	2,304

The value of process D and F becomes half respectively this table.
The processing execution time is real mechanical operation time of the necessity for the Hypotonic processing (Before injecting Fixative solution from the Hypotonic solution injection).

Note that only minus number is longer than set value (Total Hypotonic Time) at actual Hypotonic processing time.

3.23. PROCEDURE STATUS (Processes E, F, G, Gr and H)



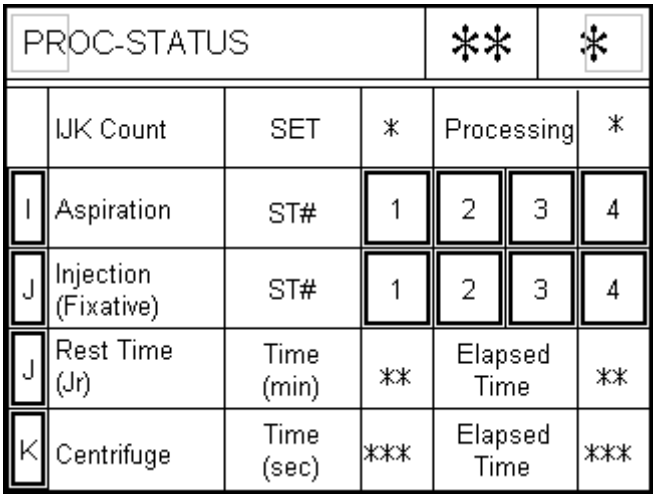
The progress in the main process will be displayed.

The name of the process (E, F, G, Gr or H) and station being executed will blink.
Once the process is complete, the display of the corresponding process will be highlighted.

The centrifuging and hypotonic times will be indicated in the count-up display from the start time.

Once the process H Centrifuging is complete, the screen will switch to (3.24) PROCEDURE STATUS (Processes I, J, and K).

3.24. PROCEDURE STATUS (Processes I, J, Jr and K)



The progress of the main process will be displayed.

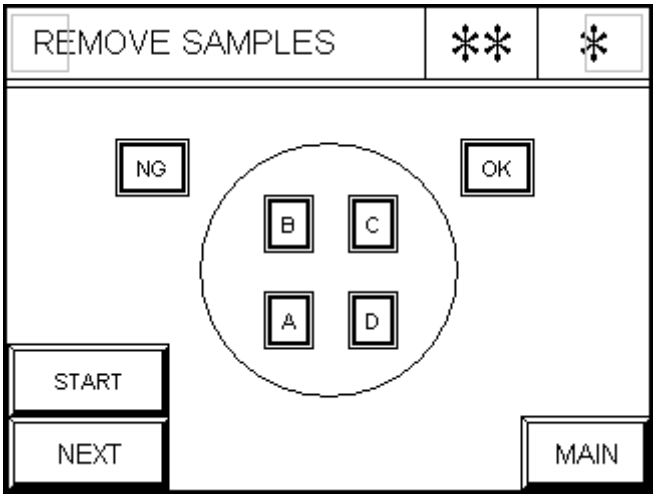
The name of the process (I, J, Jr, or K) and the station being executed will blink.
Once the process is complete, the corresponding process will be highlighted.

The centrifuging time will be indicated in the count-up display from the start time.

The IJK Process Count indicates the count number of the process currently in execution.

When the harvesting is completed, the buzzer will sound intermittently.
Subsequently, the screen will switch to (3.25) REMOVE SAMPLES.

3.25. REMOVE SAMPLES



Remove all the samples that have been set.

(1) **[Procedures]**

a) **<START>** key: **The buzzer sound will stop.**
Station #1 will be positioned at the setting position (removing position).

b) When the positioning is completed, the positioning completion lamp will light up and the buzzer will sound.

c) Open the station lid and remove all tubes in the station.

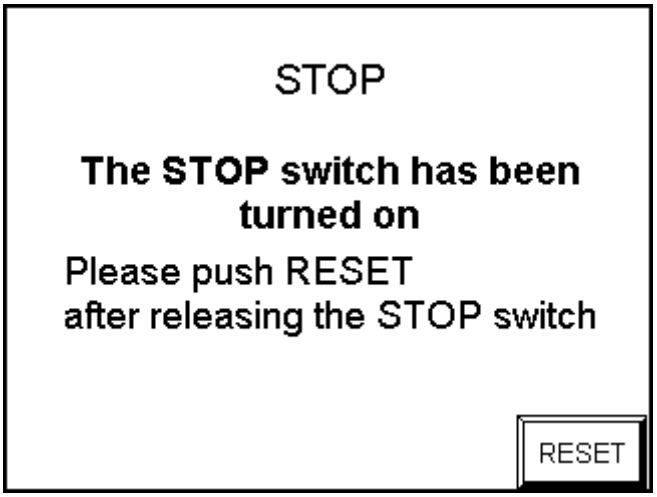
d) When [OK] is highlighted, the **<NEXT>** key will blink.

e) **<NEXT>** key: The next station will be positioned.
The station that corresponds to the set sample number will be positioned.

f) When all tubes are removed, the **<MAIN>** key will blink.

g) **<MAIN>** key: The position will descend.
The memory for the main process will be cleared.
Switches the screen to (3.1) MAIN.

3.26. STOP

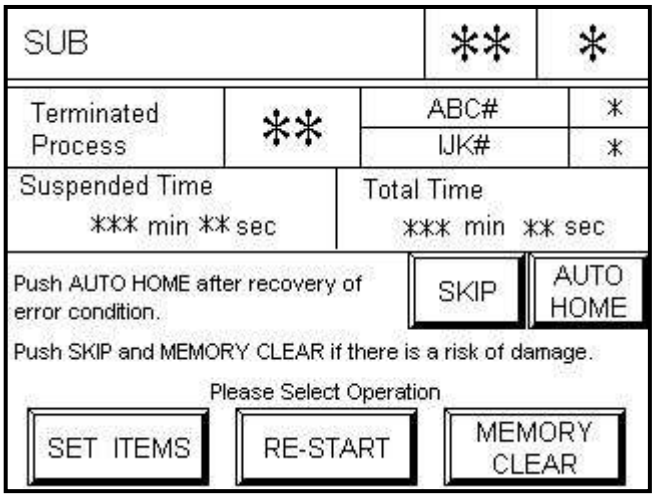


Displayed when the STOP switch is pressed.

Turn the switch clockwise to release the **STOP** switch.
The **<RESET>** key becomes effective only after the STOP switch is released.

<RESET> key: Switches the screen to (3.27) SUB.
The buzzer sound will also stop.

3.27. SUB



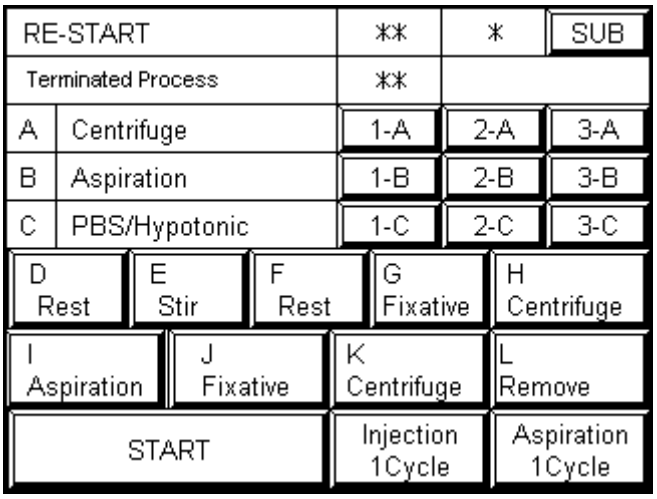
The screen for the SUB operation to be executed after the stop will appear.

(1) **[Terminated Process]**: The names of completed processes [Terminated Process] will be displayed. In addition, the completed count numbers will be displayed for repeatable processes [ABC and IJK processes].
(2) **[STOP, Error and SUB]**: When the Main, Front or Table door is opened during the main process, or an error occurs during the main process, press the RESET key, and this screen will appear.
(3) **[Suspended Time]**: Indicates the total time from start to completion of the main process.
(4) **[Total Time]**: Indicates the stop time in such cases where the main process stops due to error, etc. The process will restart from the stop time.
(Suspended Time – Total Time = Down Time)
(5) **[Power off]**: After the power is turned OFF during the main process, turn the power back ON again, and this screen will appear.

<AUTO HOME> key: Execute washing aspiration part after moving origin and become effective **<SET ITEM>**, **<MEMORY CLEAR>**, **<RE-START>** key.
<SKIP > key: Become effective **<SET ITEM>**, **<MEMORY CLEAR>**, **<RE-START>** key.
<SET ITEMS> key: Switches the screen to (3.36) SET ITEMS at SUB (Processes A1, B1 and C1).
The SUB item setting is used for the process modification when restarting.
The MAIN setting value is not changed.
<MEMORY CLEAR> key: Press and hold this key for 3 seconds to clear the terminated processes and completed count numbers.
The screen will then shift to (3.1) MAIN.
<RE-START> key: Switches the screen to (3.28) RE-START.

<SET ITEM>, **<MEMORY CLEAR>**, **<RE-START>** key aren't become effective without pushing **<AUTO HOME>** or **<SKIP>** key. Return MAIN by pushing **<SKIP>** and **<MEMORY CLEAR>** key if there is a risk of damage due to move to origin or washing aspiration part.

3.28. RE-START



This is a menu for restarting. Select the process to be restarted.
The selected process will be highlighted.
You can change the setting by newly pressing the key of the process to be restarted.

(1) **[Restarting]**

< 1-A > key: Executes A1-, B1-, C1-, A2-, B2-, C2-, A3-, B3-, and C3-processes.
< 1-B > key: Executes B1-, C1-, A2-, B2-, C2-, A3-, B3-, and C3-processes. **(*1) Caution**
< 1-C > key: Executes C1-, A2-, B2-, C2-, A3-, B3-, and C3-processes. **(*1) Caution**
< 2-A > key: Executes A2-, B2-, C2-, A3-, B3-, and C3-processes.
< 2-B > key: Executes B2-, C2-, A3-, B3-, and C3-processes. **(*1) Caution**
< 2-C > key: Executes C2-, A3-, B3-, and C3-processes. **(*1) Caution**
< 3-A > key: Executes A3-, B3-, and C3-processes.
< 3-B > key: Executes B3-, and C3-processes. **(*1) Caution**
< 3-C > key: Executes C3-processes. **(*1) Caution**

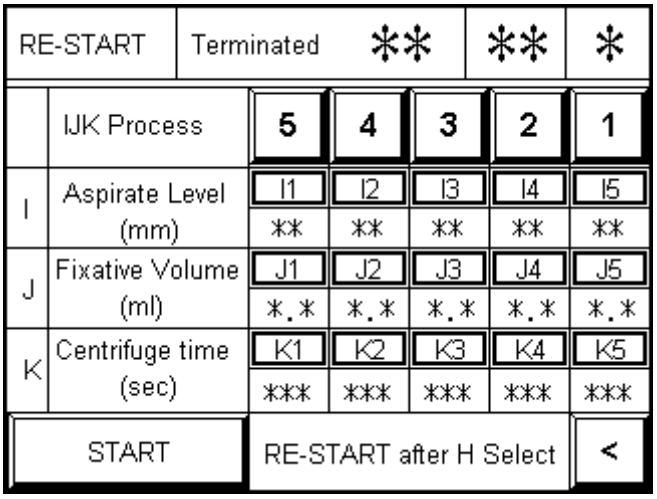
(*1)
[CAUTION]: Check the remaining solution volume in the tubes before restarting. When starting from the B-process (Aspiration), ensure the cells have sunk deeply enough before executing the process.
When starting from the C-process (Hypotonic solution injection), ensure the total volume of remaining solution in the tubes and injection volume is 7.0ml or lower.

In this case, the hypotonic time is the time where the process time from the first C-process after restarting through fixative solution injection is regulated by the total hypotonic time. To execute this process after stopping a hypotonic process, set the total hypotonic time once again.

< D Rest > key: The rest time is calculated from the setting.
< E Stir > key: Restart from E-process.
< F Rest > key: Restart from E-process. The rest time is calculated from the setting.
< G Fixative > key: **This process is not selectable if G-process is set to SKIP.**

<H Centrifuging> key: Switches the screen to (3.29) RE-START after H is selected.
<I Aspiration> key: Switches the screen to (3.30) RE-START after I is selected.
<J Injection> key: Switches the screen to (3.31) RE-START after J is selected.
<K Centrifuging> key: Switches the screen to (3.32) RE-START after K is selected.
< L Remove > key: Switches the screen to (3.25) REMOVE SAMPLES.
< START > key: Switches the screen to (3.33) CHECK TUBES.
< Injection 1 Cycle > key: Switches the screen to (3.34) INJECTION 1 CYCLE at SUB.
< Aspiration 1 Cycle > key: Switches the screen to (3.35) Aspiration 1 Cycle at SUB.
This process is not selectable if the main process is set to 1 cycle.
< SUB > key: Switches the screen to (3.27) RE-START.

3.29. RE-START after H Selected



The process count corresponding to the set items for the selected protocol will be highlighted. Set the IJK process count to be executed in RE-START after the H-process.

The connection between the set process count and operation contents is as follows:

(1) **[IJK process count setting]**
< 5 > key: Executes H, 1 (IJK), 2 (IJK), 3 (IJK), 4 (IJK), and Last (IJK).
< 4 > key: Executes H, 1 (IJK), 2 (IJK), 3 (IJK), and Last (IJK).
< 3 > key: Executes H, 1 (IJK), 2 (IJK), and Last (IJK).
< 2 > key: Executes H, 1 (IJK) and Last (IJK).
< 1 > key: Executes H and Last (IJK).
When a process count is pressed, the corresponding process contents will be highlighted.

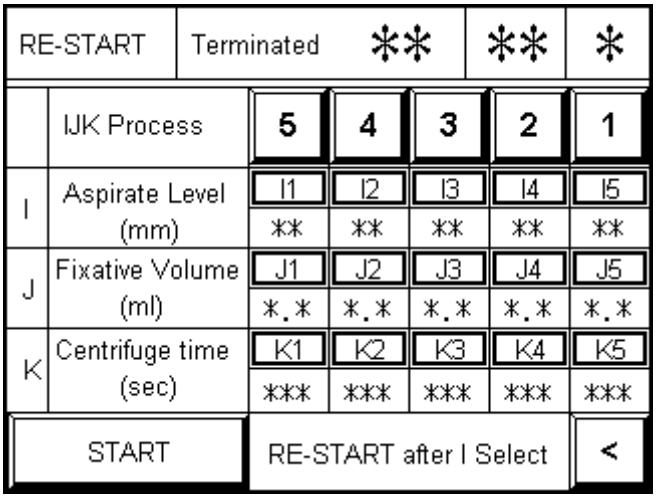
(2) **[Aspiration level]**: Displayed in height terms.
The aspiration level set in (3.13) SET ITEMS at MAIN will be displayed.
To change the settings, first return to (3.27) SUB, then change the items on the screen (3.41).

(3) **[Fixative solution injection volume]**
The injection volume set in (3.14) SET ITEMS at MAIN will be displayed.
To change the settings, first return to (3.27) the SUB screen, then change the items on the screen (3.42), and change the stirring time (3.43), the stirring timing and speed (3.44).

(4) **[Centrifuging time]**:
The centrifuging time set in (3.17) SET ITEMS at MAIN will be displayed.
To change the settings, first return to (3.27) SUB, then change the items on the screen (3.45).

<START> key: Switches the screen to (3.33) CHECK TUBES.
< ◀ **>** key: Switches the screen to (3.28) RE-START.

3.30. RE-START after I Selected



The process count corresponding to the set items for the selected protocol will be highlighted.
Set the IJK process count to be executed in RE-START after the I-process.
The operation will be executed from the I-process.

Check that samples have been sufficiently centrifuged.

The connection between the set process count and operation contents is as follows:

(1) **[IJK process count setting]**
< 5 > key: Executes 1 (IJK), 2 (IJK), 3 (IJK), 4 (IJK), and Last (IJK).
< 4 > key: Executes 1 (IJK), 2 (IJK), 3 (IJK), and Last (IJK).
< 3 > key: Executes 1 (IJK), 2 (IJK), and Last (IJK).
< 2 > key: Executes 1 (IJK) and Last (IJK).
< 1 > key: Executes Last (IJK).
When a process count is pressed, the corresponding process contents will be highlighted.

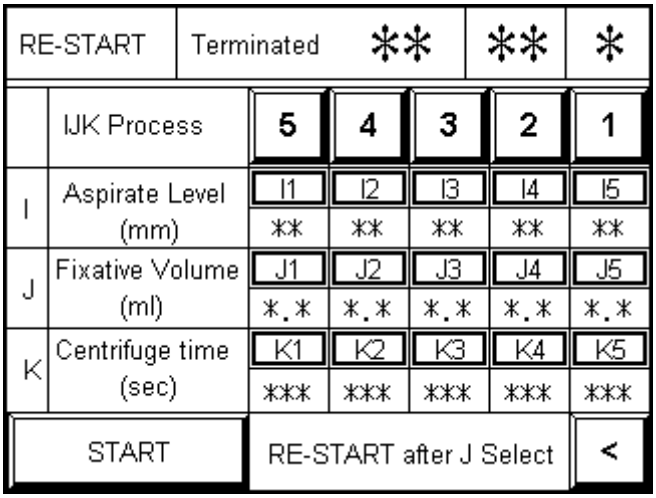
(2) **[Aspiration level]**: Displayed in height terms.
The aspiration level set in (3.13) SET ITEMS at MAIN will be displayed.
To change the settings, first return to (3.27) SUB, then change the items on the screen (3.41).

(3) **[Fixative solution injection volume]**
The injection volume set in (3.14) SET ITEMS at MAIN will be displayed.
To change the settings, first return to (3.27) the SUB screen, then change the items on the screen (3.42), and change the stirring time (3.43), the stirring timing and speed (3.44).

(4) **[Centrifuging time]**:
The centrifuging time set in (3.17) SET ITEMS at MAIN will be displayed.
To change the settings, first return to (3.27) SUB, then change the items on the screen (3.45).

<START> key: Switches the screen to (3.33) CHECK TUBES.
< ◀ **>** key: Switches the screen to (3.28) RE-START.

3.31. RE-START after J Selected



The process count corresponding to the set items for the selected protocol will be highlighted.
Set the IJK process count to be executed in RE-START after the J-process.
The operation will be executed from the J-process.

[CAUTION]
If remaining the solution in the tube after injection is set more than 7.0ml, it may spatter during stirring, result in serious damage to the equipment. The solution volume level in the tube should therefore be set with due caution.

The connection between the set process count and operation contents is as follows:

(1) **[IJK process count setting]**
< 5 > key: Executes 1 (JK), 2 (IJK), 3 (IJK), 4 (IJK), and Last (IJK).
< 4 > key: Executes 1 (JK), 2 (IJK), 3 (IJK), and Last (IJK).
< 3 > key: Executes 1 (JK), 2 (IJK), and Last (IJK).
< 2 > key: Executes 1 (JK) and Last (IJK).
< 1 > key: Executes Last (JK).
When a process count is pressed, the corresponding process contents will be highlighted.

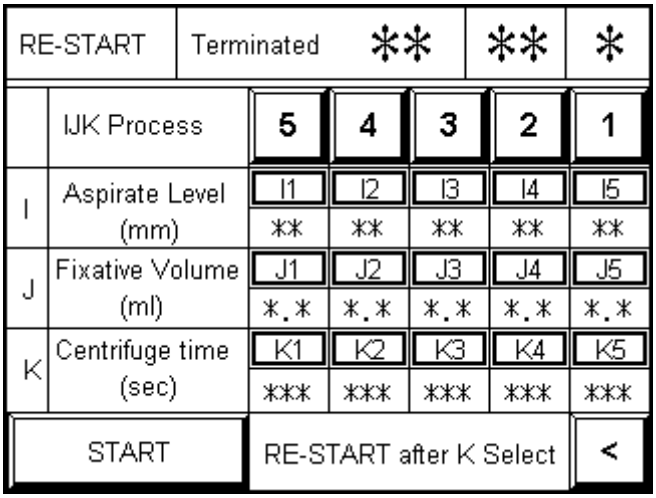
(2) **[Aspiration level]**: Displayed in height terms.
The aspiration level set in (3.13) SET ITEMS at MAIN will be displayed.
To change the settings, first return to (3.27) SUB, then change the items on the screen (3.41).

(3) **[Fixative solution injection volume]**
The injection volume set in (3.14) SET ITEMS at MAIN will be displayed.
To change the settings, first return to (3.27) the SUB screen, then change the items on the screen (3.42), and change the stirring time (3.43), the stirring timing and speed (3.44).

(4) **[Centrifuging time]**:
The centrifuging time set in (3.17) SET ITEMS at MAIN will be displayed.
To change the settings, first return to (3.27) SUB, then change the items on the screen (3.45).

<START> key: Switches the screen to (3.33) CHECK TUBES.
< ◄ **>** key: Switches the screen to (3.28) RE-START.

3.32. RE-START after K Selected



The process count corresponding to the set items for the selected protocol will be highlighted.
Set the IJK process count to be executed in RE-START after the K-process.
The operation will be executed from the K-process.

The connection between the set process count and operation contents is as follows:

(1) **[IJK process count setting]**
< 5 > key: Executes 1 (K), 2 (IJK), 3 (IJK), 4 (IJK), and Last (IJK).
< 4 > key: Executes 1 (K), 2 (IJK), 3 (IJK), and Last (IJK).
< 3 > key: Executes 1 (K), 2 (IJK), and Last (IJK).
< 2 > key: Executes 1 (K) and Last (IJK).
< 1 > key: Executes Last (K).
When a process count is pressed, the corresponding process contents will be highlighted.

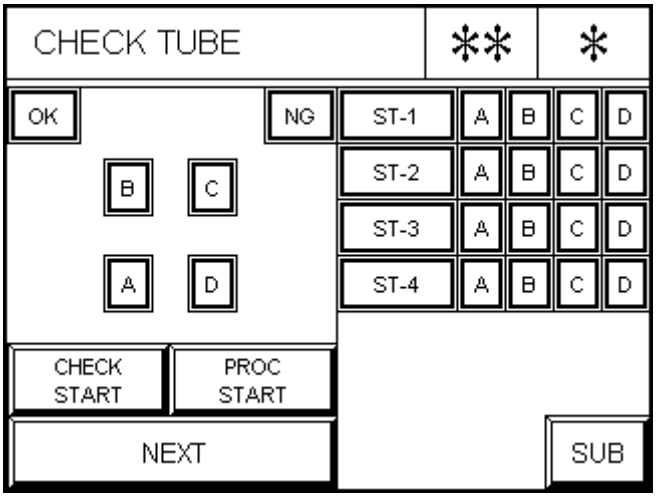
(2) **[Aspiration level]**: Displayed in height terms.
The aspiration level set in (3.13) SET ITEMS at MAIN will be displayed.
To change the settings, first return to (3.27) SUB, then change the items on the screen (3.41).

(3) **[Fixative solution injection volume]**
The injection volume set in (3.14) SET ITEMS at MAIN will be displayed.
To change the settings, first return to (3.27) the SUB screen, then change the items on the screen (3.42), and change the stirring time (3.43), the stirring timing and speed (3.44).

(4) **[Centrifuging time]**:
The centrifuging time set in (3.17) SET ITEMS at MAIN will be displayed.
To change the settings, first return to (3.27) SUB, then change the items on the screen (3.45).

<START> key: Switches the screen to (3.33) CHECK TUBES.
< ◄ **>** key: Switches the screen to (3.28) RE-START.

3.33. CHECK the TUBES



If an operation is started on RE-START (3.28), (3.29), (3.30), (3.31), or (3.32), the screen will shift to this screen. A, B, C and D of the station to be set will be highlighted.

Check that the tubes are set properly for all stations.

(1) **[Procedures]**

a) **<CHECK START>** key: Station #1 will be positioned at the setting position.

b) When the positioning is completed, the positioning completion lamp will light up and the buzzer will sound.
Do not touch the station before the lamp lights up and the buzzer sounds.
If you do so, it may result in a positioning failure.
The sensor checks that the number of samples set corresponds to the number of samples selected.
-> When they match, [**OK**] will be highlighted.
-> If not, [**NG**] will be highlighted.
If [**NG**] is highlighted, remove the tube that has been set so that [**OK**] will be highlighted.

c) Open the station lid. **Be sure to set four tubes, and then close the lid securely.**

If the lid is not closed securely, samples will be insufficiently stirred, which may result in a harvesting failure.

d) When [**OK**] is highlighted, the **<NEXT>** key will blink.

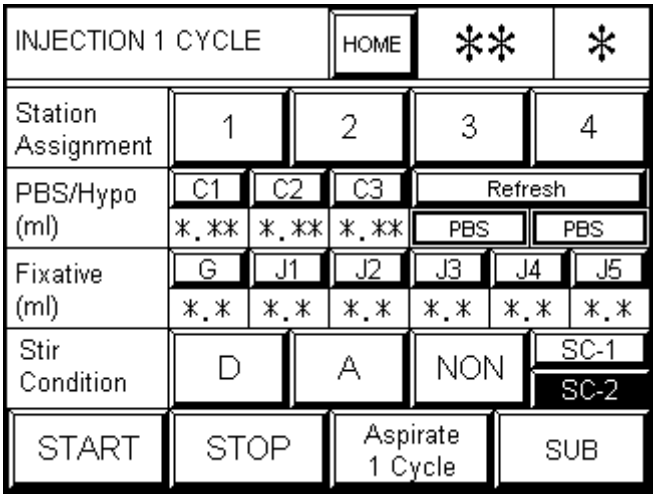
e) **<NEXT>** key: The next station will be positioned.

f) When all four stations are checked, the **<START>** key will blink.

g) **<PROC START>** key: Press and hold the key for 3 seconds to start operation.
If the instruction is accepted, the buzzer will sound and the screen will change.

< SUB > key: Switches the screen to (3.27) SUB.

3.34. INJECTION 1 CYCLE at RE-START



There is no default setting. Three settings (Station #, PBS/Hypotonic or fixative solution, and stirring condition) must be specified before starting an operation.
The selected keys will be highlighted until the screen shifts to another.
Solution will be injected into 4 tubes by each station.

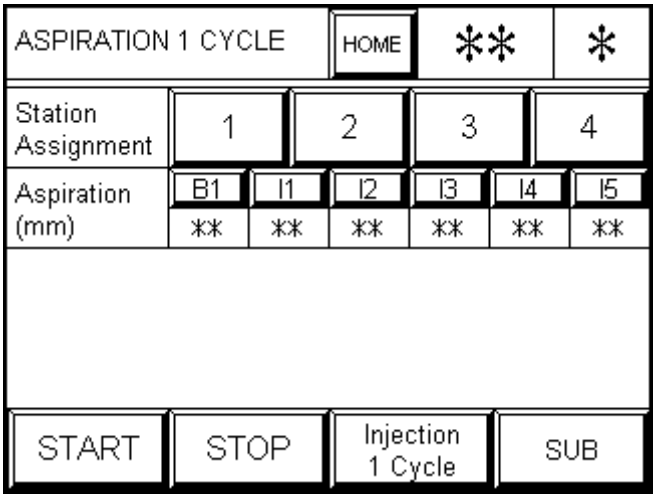
(1) **[Station assignment]**:
The selectable stations are those corresponding to the set number for the main process.

(2) **[Specification of solution]**: Select either PBS/hypotonic or Fixative solution.
The solution volume corresponding to each protocol will be highlighted.
The setting values can be changed from [Set Items] on the (3.27) RE-START screen.

(3) **[Stirring]**
< D > key: PBS/Hypotonic or fixative solution is injected during stirring.
< A > key: PBS/Hypotonic or fixative solution is injected after stirring.
<NON> key: Only the injection is executed. No stirring is executed.
<SC-1> key: Speed conrol 1.
<SC-2> key: Speed conrol 2.

<START> key: The Station, PBS/hypotonic or Fixative solution, and stirring condition must be specified before starting an operation.
<STOP> key: The operation will stop instantly. This key is effective during a 1-cycle process.
<Aspiration 1 Cycle> key: Switches the screen to (3.35) ASPIRATION 1 CYCLE at RE-START.
< SUB > key: Switches the screen to (3.27) SUB.
<HOME> key: The position will return to the original point.
If the sensor fails to confirm that operating parts are positioned at the original point, the display will be highlighted.
Unless all the operating parts return to the original point, the equipment cannot proceed to the next operation.

3.35. ASPIRATION 1 CYCLE at RE-START



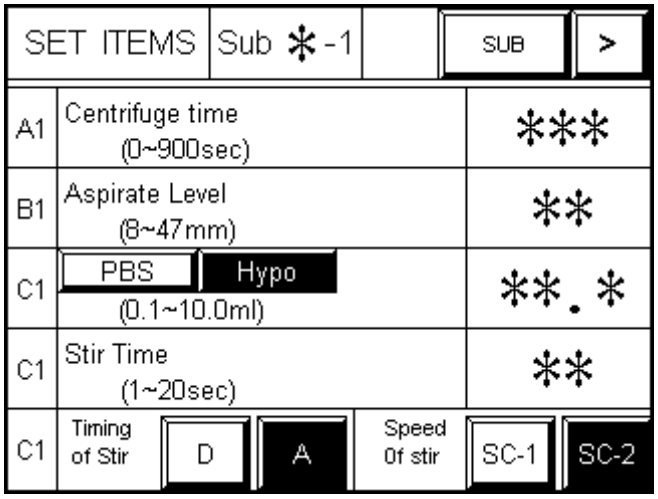
There is no default setting. You must specify two settings (Station # and aspiration level B, I1, I2, I3, I4 or I5) before starting an operation.
Selected keys will be highlighted until the screen shifts to another.
Solution will be removed from all 4 tubes by each station.

(1) **[Station assignment]**: Selectable stations are those that correspond to set number for the main process.

(2) **[Level selection]** Select the aspiration level.
The aspiration level that corresponds to each protocol will be highlighted.
The setting values can be changed from [Set Items] on (3.27) SUB screen.

<START> key: You must select the Station # and Aspiration Level B, I1, I2, I3, I4 or I5 before starting an operation.
<STOP> key: The operation will stop instantly. This key is effective during 1-cycle process.
<Injection 1 Cycle> key: Switches the screen to (3.34) INJECTION 1 CYCLE at RE-START.
< SUB > key: Switches the screen to (3.27) SUB.
<HOME> key: The position will return to the original point.
If the sensor fails to confirm that operating parts are positioned at the original point, the display will be highlighted.
Unless all the operating parts return to the original point, the equipment cannot proceed to the next operation.

3.36. SET ITEMS at SUB (Processes A1, B1, and C1)



First ABC process setting: Set the centrifuging time for the A-process, aspiration level (B) for the B-process, PBS/hypotonic solution injection volume (C) and stirring time/timing/speed for the C-process.

V [ml]	H [mm]
0.15	8
0.2	9
0.3	11
0.5	14
1.0	19
1.5	23
2.0	27
2.5	30
3.0	34
3.5	37
4.0	40
4.5	44
5.0	47

(1) **[Centrifuging time]**

The setting time includes the rise time (about 18 seconds), but not the fall time (warm-down time, about 25 seconds).

The air bath control is turned off while centrifuging.

(2) **[Aspiration level]**: Displayed in height terms.

Setting range: 47mm = 5.0ml level
8mm = 0.15ml level

(3) **[Hypotonic solution injection volume]**

The value can be set at intervals of 0.1ml.

Set the value such that the total volume of the solution volume remaining in the tube at the aspiration level (B), hypotonic solution injection volume (C), and fixative solution injection volume (G) in G-process becomes 7.0ml or less.

Example: B=1.0ml, C=5.5ml, G=0.5ml TOTAL: 7.0ml

[CAUTION]
If remaining the solution in the tube after injection is set more than 7.0ml, it may spatter during stirring, result in serious damage to the equipment. The solution volume level in the tube should therefore be set with due caution.

(4) **[Hypotonic solution stirring time]**

The stirring rate increases in two stages The rotation speed is a half of the set speed for the first 1 second.
The stir timing can be set to two ways. These are D(Stir during injection) and A(Stir after injection). The stir speed can be set to two stages (SC-1, SC-2). The stir speed setting opens and adjusts a front right door.
(Refer to the photograph.)

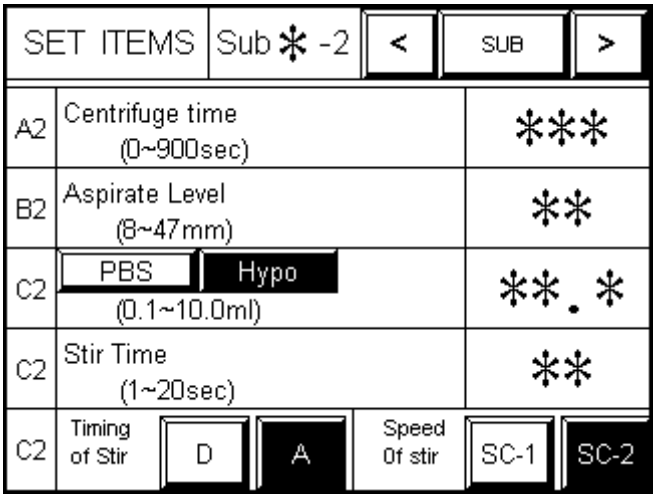
(5) **[How to change the setting values]**

Touch the setting value display, ten key is displayed.
Change the value using the numeric keypad and confirm with the ENT key. The numbers other than the range of the setting cannot be input.

< SUB > key: Switches the screen to (3.27) SUB.
< ▶ **>** key: Switches the screen to (3.37) SET ITEMS at SUB (Processes A2, B2, and C2).

*** The previously set values are stored for each setting value and displayed when each setting screen appears.**
*** The values in parentheses indicate the adjustable range. Any value outside this range is unacceptable.**

3.37. SET ITEMS at SUB (Processes A2, B2, and C2)



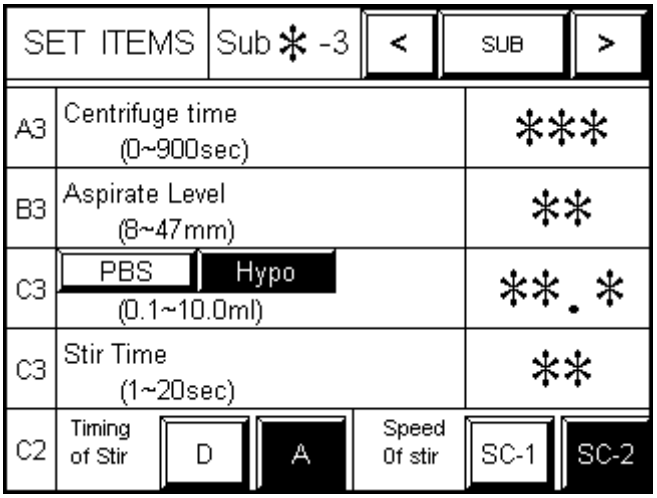
Second ABC process setting: Set the centrifuging time for the A-process, aspiration level (B) for the B-process, PBS/Hypotonic solution injection volume (C) and stirring time/timing/speed for the C-process.

The setting method is the same as (3.36).

(1) **[How to change the setting values]**
Touch the setting value display, ten key is displayed.
Change the value using the numeric keypad and confirm with the ENT key. The numbers other than the range of the setting cannot be input.

< SUB > key: Switches the screen to (3.27) SUB.
< ▶ **>** key: Switches the screen to (3.38) SET ITEMS at SUB (Processes A2, B2, and C2).
< ◀ **>** key: Switches the screen to (3.36) SET ITEMS at SUB (Processes A1, B1, and C1).

3.38. SET ITEMS at SUB (Processes A3, B3, and C3)



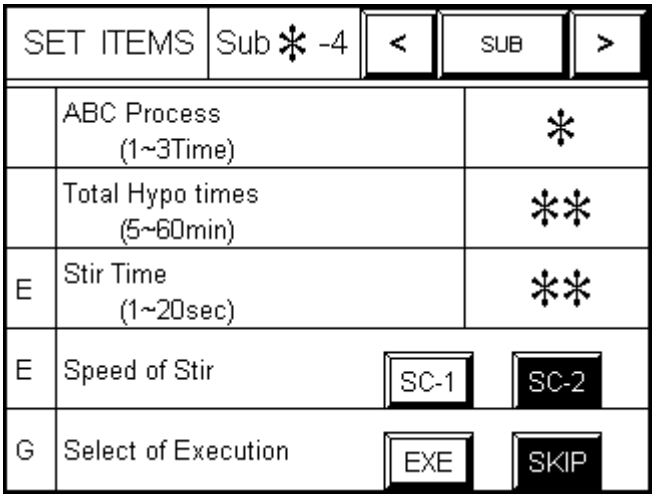
Third ABC process setting: Set the centrifuging time for the A-process, aspiration level (B) for the B-process, PBS/Hypotonic solution injection volume (C) and stirring time/timing/speed for the C-process.

The setting method is the same as (3.36).

(1) **[How to change the setting values]**
Touch the setting value display, ten key is displayed.
Change the value using the numeric keypad and confirm with the ENT key. The numbers other than the range of the setting cannot be input.

< SUB > key: Switches the screen to (3.27) SUB.
< ▶ **>** key: Switches the screen to (3.39) SET ITEMS at SUB (Processes E and G).
< ◀ **>** key: Switches the screen to (3.37) SET ITEMS at SUB (Processes A2, B2, and C2).

3.39. SET ITEMS at SUB (Processes E and G)



Set the ABC process count, total hypotonic time, stirring time/timing/speed for the E-process for the hypotonic rest and select of the G-process execute/skip.

(1) **[ABC process count]**
Tow counts = A, B, and C processes will be executed twice.
The maximum, set value is three.

(2) **[Total hypotonic time]**
Enter the total hypotonic time between 5 to 60 minutes. Total hypotonic time is setting 20 minutes or more. Total hypotonic processing time is defined from hypotonic solution injection (C2 when PBS is injected with C1) at the time of the first fixative solution injection (G or J1). Therefore, the rest time changes by the number of ABC execution and G process skip.

* **[Hypotonic Rest Time]**
Please refer to the calculation type at the Rest time of the hypotonic solution for (3.22) Main process progress (A,B,C,D) for the Rest time.
However, the Rest time of D and F becomes 0 seconds at the following setting.
(a) ABC=1 & C1=PBS
(b) ABC=2 & C1=C2=PBS
(c) ABC=3 & C1=C2=C3=PBS

(3) **[Stirring Time]** Setting of E process stir time

(4) **[Stir Speed]** The stir speed can be set to two stages (SC-1, SC-2).

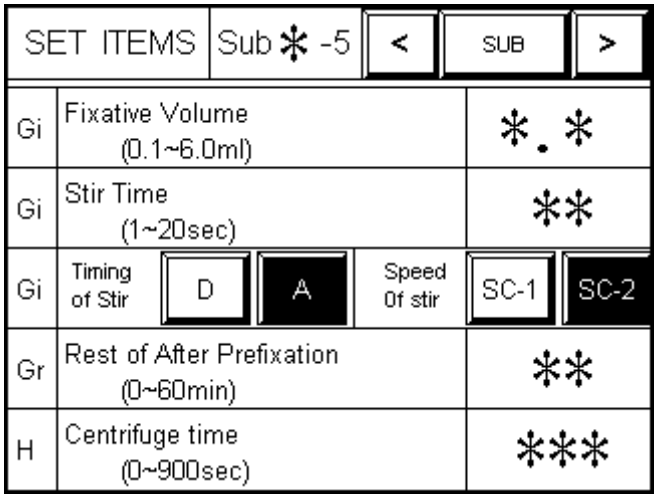
(5) **[G Select of Execution]**
< EXE > key: The G-process will be executed.
< SKIP > key: The G-process will not be executed.

When G process is skipped, fixative solution will be injected for the first time in the process (J1) through centrifuge (H) and process (I1).

(6) **[How to change the setting values]**
Touch the setting value display, ten key is displayed.
Change the value using the numeric keypad and confirm with the ENT key. The numbers other than the range of the setting cannot be input.

< SUB > key: Switches the screen to (3.27) SUB.
< ▶ **>** key: Switches the screen to (3.40) SET ITEMS at SUB (Process G and H).
< ◀ **>** key: Switches the screen to (3.38) SET ITEMS at SUB (Processes A3, B3, and C3).

3.40. SET ITEMS at SUB (Processes G and H)



Set injection volume of the first fixative solution, stirring time/timing/speed for the G-process, rest time of the first fixative, and centrifuge time for the H-process.

(1) **[Fixative solution injection volume (G)]**
The value can be set at intervals of 0.1ml.

Set the value such that the total volume of the solution volume remaining in the tube at the aspiration level (B), hypotonic solution injection volume (C), and fixative solution injection volume (G) in G-process becomes 7.0ml or less.

Example: B=1.0ml, C=5.5ml, G=0.5ml TOTAL: 7.0ml

[CAUTION]
If remaining the solution in the tube after injection is set more than 7.0ml, it may spatter during stirring, result in serious damage to the equipment. The solution volume level in the tube should therefore be set with due caution.

(2) **[Stirring time] for G-process**

(3) **[Rest time] for G-process**
Rest time can set it from 0 to 60 minutes. The zero out doesn't execute rest time.

(4) **[Centrifuging time]**
The setting time includes the rise time (about 18 seconds),
but not the fall time (warm-down time, about 25 seconds).

(5) **[How to change the setting values]**
Touch the setting value display, ten key is displayed.
Change the value using the numeric keypad and confirm with the ENT key. The numbers other than the range of the setting cannot be input.

< SUB > key: Switches the screen to (3.27) SUB.
< ▶ **>** key: Switches the screen to (3.41) SET ITEMS at SUB (Process I).
< ◀ **>** key: Switches the screen to (3.39) SET ITEMS at SUB (Processes E and G).

3.41. SET ITEMS at SUB (Process I)

SET ITEMS	Sub ✻ -6	<	SUB	>
I1	Aspirate Level (8~47mm)	✻✻		
I2	Aspirate Level (8~47mm)	✻✻		
I3	Aspirate Level (8~47mm)	✻✻		
I4	Aspirate Level (8~47mm)	✻✻		
I5	Aspirate Level (8~47mm)	✻✻		

Set the aspiration levels I1, I2, I3, I4 and I5 for the I-process.

(1) **[Aspiration level]:**

A point inside in the sample tube is zero levels. The setting is units of mm in the direction of height.
It is not height from a mechanical starting point of the needle for suction.

Setting conditions for the I-process aspiration level (I1):
When the J-process fixative solution injection volume (J1)
is 5.0ml: Shall be set to 27 or lower.
For 2.0ml or less: No limitation in setting value.

V [ml]	H [mm]
0.15	8
0.2	9
0.3	11
0.5	14
1.0	19
1.5	23
2.0	27
2.5	30
3.0	34
3.5	37
4.0	40
4.5	44
5.0	47

[CAUTION]
If remaining in the tube after injection is set more than 7.0ml, it may spatter during stirring, result in serious damage to the equipment. The solution volume level in the tube should therefore be set with due caution.

(2) **[How to change the setting values]**

Touch the setting value display, ten key is displayed.
Change the value using the numeric keypad and confirm with the ENT key. The numbers other than the range of the setting cannot be input.

< SUB > key: Switches the screen to (3.27) SUB.
< ▶ **>** key: Switches the screen to (3.42) SET ITEMS at SUB (Process J).
< ◀ **>** key: Switches the screen to (3.40) SET ITEMS at SUB (Processes G and H).

3.42. SET ITEMS at SUB (Process J)

SET ITEMS	Sub ✻-7	<	SUB	>
J1	Fixative Volume (0.1~6.0ml)	✻.✻		
J2	Fixative Volume (0.1~6.0ml)	✻.✻		
J3	Fixative Volume (0.1~6.0ml)	✻.✻		
J4	Fixative Volume (0.1~6.0ml)	✻.✻		
J5	Fixative Volume (0.1~6.0ml)	✻.✻		

Set the fixative solution injection volumes (J1, J2, J3, J4 and J5) for the J-process.

(1) **[Fixative solution injection volume]**

The value can be set at intervals of 0.1ml.

J-process fixative solution injection volume (J1):

When the I-process aspiration level (I1) is 40mm: Shall be set to 3.0ml or lower.

For the level (I1) of 19 or lower: No limitation in setting value.

[CAUTION]

If remaining the solution in the tube after injection is set more than 7.0ml, it may spatter during stirring, result in serious damage to the equipment. The solution volume level in the tube should therefore be set with due caution.

(2) **[How to change the setting values]**

Touch the setting value display, ten key is displayed.

Change the value using the numeric keypad and confirm with the ENT key. The numbers other than the range of the setting cannot be input.

< SUB > key: Switches the screen to (3.27) SUB.

< ▶ **>** key: Switches the screen to (3.43) SET ITEMS at SUB (Processes J).

< ◀ **>** key: Switches the screen to (3.41) SET ITEMS at SUB (Process I).

3.43. SET ITEMS at SUB (Process J)

SET ITEMS	Sub ✻ -8	<	SUB	>
J1	Stir Time (1~20sec)	✻✻		
J2	Stir Time (1~20sec)	✻✻		
J3	Stir Time (1~20sec)	✻✻		
J4	Stir Time (1~20sec)	✻✻		
J5	Stir Time (1~20sec)	✻✻		

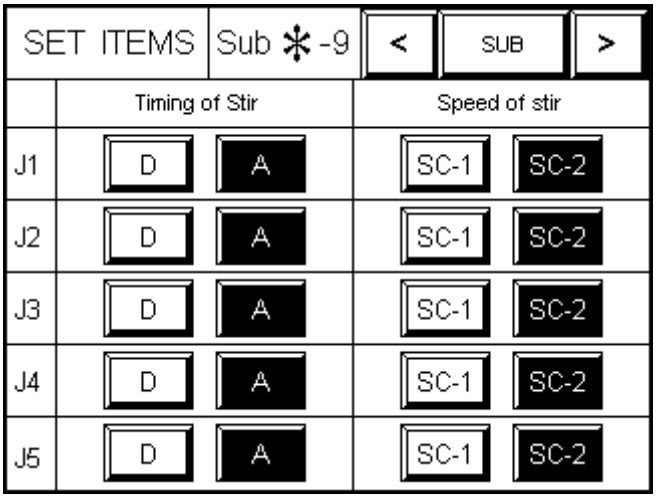
Set the fixative solution stirring time (J1, J2, J3, J4 and J5) for the J-process.

(1) **[Fixative solution stirring time]**
The stir time can be set respectively of J1-J5.

(2) **[How to change the setting values]**
Touch the setting value display, ten key is displayed.
Change the value using the numeric keypad and confirm with the ENT key. The numbers other than the range of the setting cannot be input.

< SUB > key: Switches the screen to (3.27) SUB.
< ▶ **>** key: Switches the screen to (3.44) SET ITEMS at SUB (Processes J).
< ◀ **>** key: Switches the screen to (3.42) SET ITEMS at SUB (Process I).

3.44. SET ITEMS at SUB (Process J)



Set the fixative solution stirring timing and speed (J1, J2, J3, J4 and J5) for the J-process.

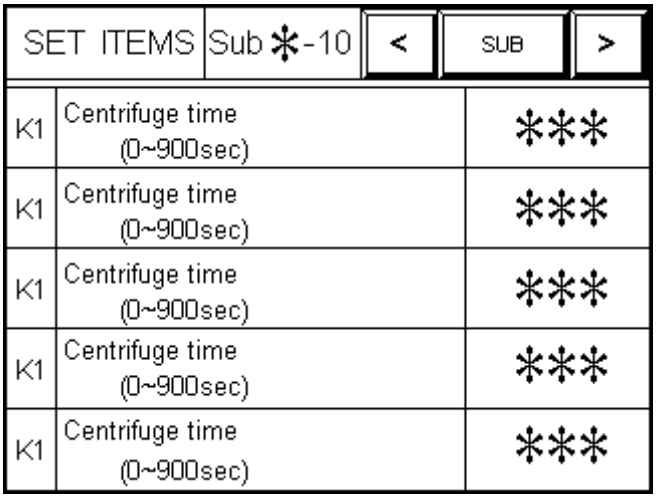
(1) **[Fixative solution stirring timing]**
The stir timing can be set to two ways D (During) and A (After).

(2) **[Fixative solution stirring speed]**
The stir speed can be set to two stages (SC-1, SC-2).

(3) **[How to change the setting values]**
Touch the setting value display, ten key is displayed.
Change the value using the numeric keypad and confirm with the ENT key. The numbers other than the range of the setting cannot be input.

< SUB > key: Switches the screen to (3.27) SUB.
< ▶ **>** key: Switches the screen to (3.45) SET ITEMS at SUB (Processes K).
< ◀ **>** key: Switches the screen to (3.43) SET ITEMS at SUB (Process J).

3.45. SET ITEMS at SUB (Process K)



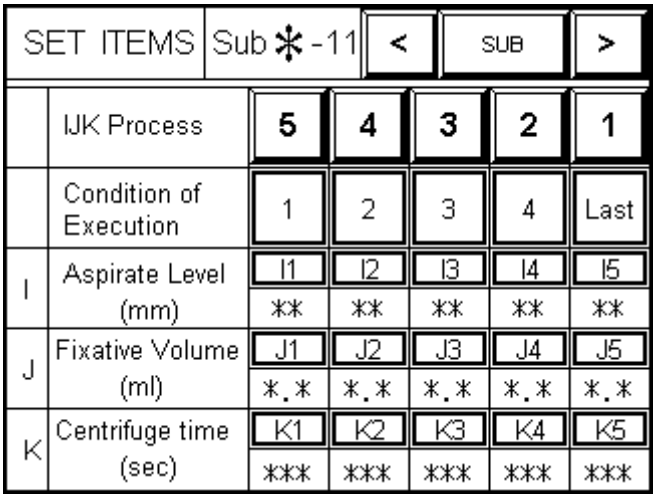
Set the fixative solution centrifuge time (K1, K2, K3, K4 and K5) for the K-process.

(1) **[Centrifuging time]**
The setting time includes the rise time (about 18 seconds), but not the fall time (warm-down time, about 25 seconds.)

(2) **[How to change the setting values]**
Touch the setting value display, ten key is displayed.
Change the value using the numeric keypad and confirm with the ENT key. The numbers other than the range of the setting cannot be input.

< SUB > key: Switches the screen to (3.27) SUB.
< ▶ **>** key: Switches the screen to (3.46) SET ITEMS at SUB (Processes I,J,K).
< ◀ **>** key: Switches the screen to (3.44) SET ITEMS at SUB (Process J).

3.46. SET ITEMS at SUB (Processes I, J and K)



Set the IJK process count.
The connection between the set process count and operation contents is as follows:

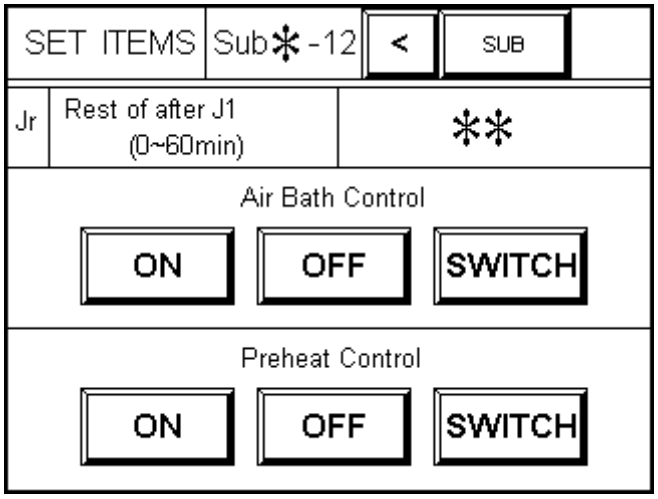
(1) **[IJK process count setting]**
< 5 > key: Executes 1 (IJK), 2 (IJK), 3 ((IJK), 4 ((IJK), and Last ((IJK).
< 4 > key: Executes 1 (IJK), 2 (IJK), 3 ((IJK), and Last ((IJK).
< 3 > key: Executes 1 (IJK), 2 (IJK), and Last ((IJK).
< 2 > key: Executes 1 (IJK) and Last ((IJK).
< 1 > key: Executes Last ((IJK).

When a process count is pressed, the corresponding process contents will be highlighted.

(2) **[How to change the setting values]**
Touch the setting value display, ten key is displayed.
Change the value using the numeric keypad and confirm with the ENT key. The numbers other than the range of the setting cannot be input.

< SUB > key: Switches the screen to (3.27) SUB.
< ▶ **>** key: Switches the screen to (3.47) SET ITEMS at SUB (Processes Jr).
< ◀ **>** key: Switches the screen to (3.45) SET ITEMS at SUB (Processes K).

3.47. SET ITEMS at SUB (Processes Jr)



Set the Jr rest time of after J1, air bath control mode and preheat control mode.

(1) **[Rest time] for after the J1process**
Rest time can set it from 0 to 60 minutes. The zero out doesn't execute rest time.

(2) **[Air bath Control mode]**
< ON > key: The temperature is continuously controlled while the power is on.
< OFF > key: The air bath temperature control is turned OFF.
<SWITCH> key: The temperature control is turned off from the G-process due to the sample removal.

(3) **[Preheat Control mode]**
< ON > key: The temperature is continuously controlled while the power is on.
< OFF > key: The preheat temperature control is turned OFF.
<SWITCH> key: The temperature control is turned off from the G-process due to the sample removal.

(4) **[How to change the setting values]**
Touch the setting value display, ten key is displayed.
Change the value using the numeric keypad and confirm with the ENT key. The numbers other than the range of the setting cannot be input.

< SUB > key: Switches the screen to (3.27) SUB.
< ◀ **>** key: Switches the screen to (3.46) SET ITEMS at SUB (Processes I,J,K).

3.48. WARNING (Centrifuge Door Open)

If the Centrifuge Door is opened starting the main process, the process will be stopped. Then the alarm will sound and the screen will be switched.

< RESET > key: Switches the screen to (3.21) SET SAMPLES.
The alarm sound will stop.

The next operation instruction is not accepted until the door is closed.

3.49. WARNING (Main Door Open)

If the Main Door is opened starting the main process, the process will be stopped. Then the alarm will sound and the screen will be switched.

< RESET > key: Switches the screen to (3.21) SET SAMPLES.
The alarm sound will stop.

The next operation instruction is not accepted until the door is closed.

3.50. WARNING (Front Door Open)

If the Front Door is opened starting the main process, the process will be stopped. Then the alarm will sound and the screen will be switched.

< RESET > key: Switches the screen to (3.21) SET SAMPLES.
The alarm sound will stop.

The next operation instruction is not accepted until the door is closed.

3.51. WARNING (Rear Door Open)

If the Rear Door is opened starting the main process, the process will be stopped. Then the alarm will sound and the screen will be switched.

< RESET > key: Switches the screen to (3.21) SET SAMPLES.
The alarm sound will stop.

The next operation instruction is not accepted until the door is closed.

3.52. CAUTION (Main Door Open)

If the Main Door is opened during the main process, the process will be stopped.
Then the alarm will sound and the screen will be switched.

< RESET > key: Switches the screen to (3.27) SUB.
The alarm sound will stop.

The next operation instruction is not accepted until the door is closed.

A Main Door mounts the switch of the mechanical safety lock.
The door cannot be opened while executing Main Processing usually.

3.53. CAUTION (Front Door Open)

If the Front Door is opened during the main process, the process will be stopped. Then the alarm will sound and the screen will be switched.

< RESET > key: Switches the screen to (3.27) SUB.
The alarm sound will stop.

The next operation instruction is not accepted until the door is closed.

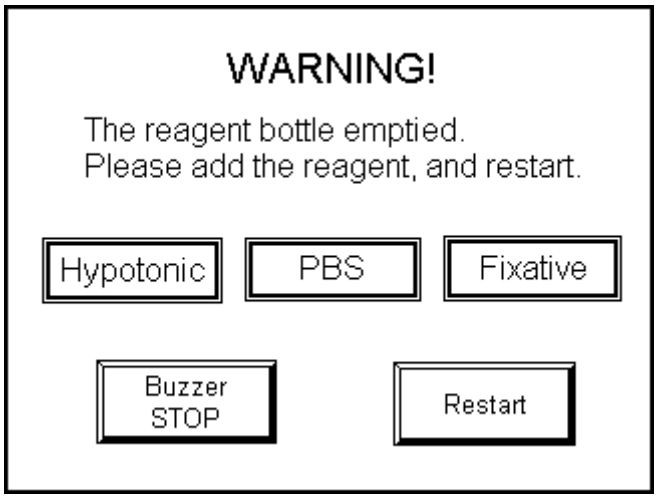
3.54. CAUTION (Rear Door Open)

If the Rear Door is opened during the main process, the process will be stopped.
Then the alarm will sound and the screen will be switched.

< RESET > key: Switches the screen to (3.27) SUB.
The alarm sound will stop.

The next operation instruction is not accepted until the door is closed.

3.55. Warning (Reagent Bottle Empty)

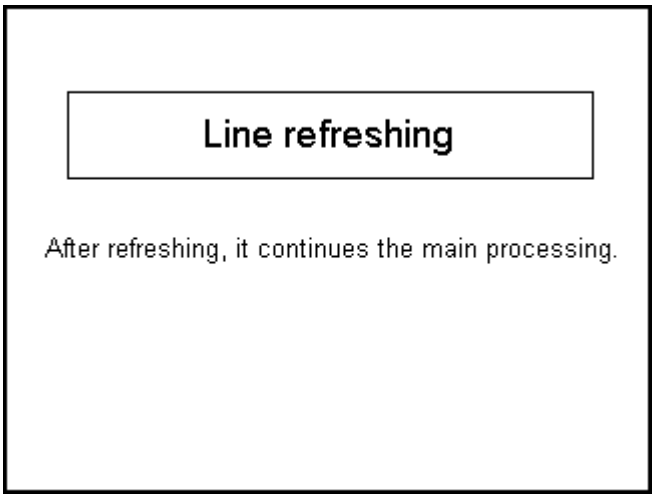


This process is interrupted, and the screen switches with the cautioning alarm sound when the reagent disappears in this process.
(The key to the main door is not open lock.)

Push the restart after refilling the reagent.

The highlight character is displayed to the detected reagent (either Hypotonic solution, PBS or Fixative).
The processing of the interruption is continued by the restart automatically.
(The automatic continuance processing is not made when interrupted again by other cautioning etc.)

Action flow of automatic continuance
(a) An empty reagent is detected during the injection action.
(b) The injection of the amount is completed once after the reagent is empty detected and it stops.
(c) **< Buzzer STOP >** key is pushed, the reagent is refilled, and **< Restart >** key is pushed.
(d) The reagent is refreshed, and processing from the interrupting following is continued.



4. Specifications

This equipment is used to carry out harvesting (PBS/Hypotonic and fixative operation) of cultured cell suspensions.

4.1. Main specifications

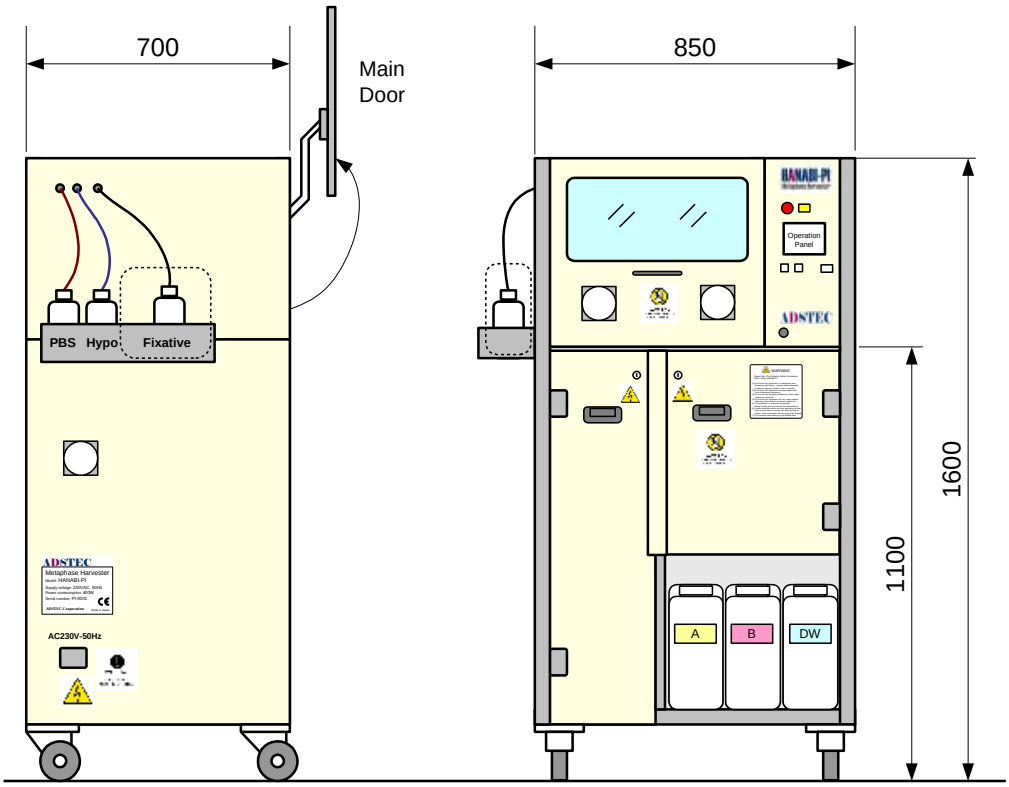
HANABI-PI Metaphase Harvester Specifications

2010.09.06

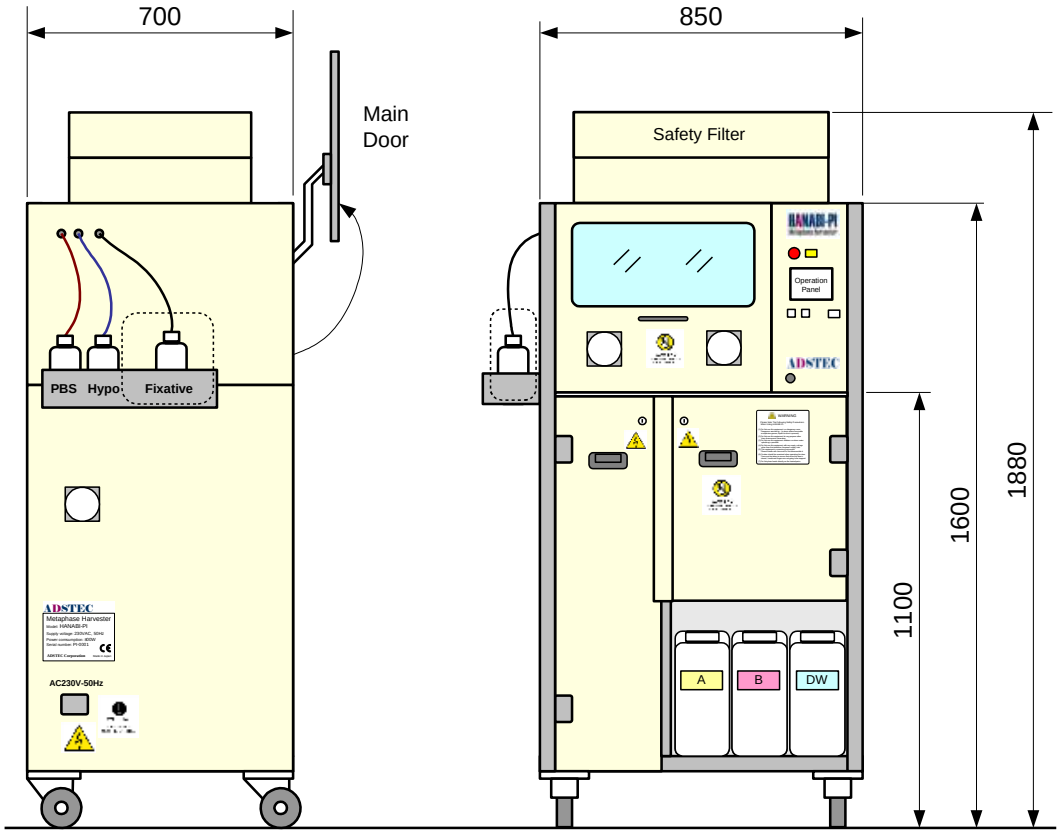
#	Items	Specifications	Remarks
1	Centrifugal tube	15 ml	FALCON (Polypropylene)
2	Culture cell suspensions	10 ml (Max.)	Typical 5ml
3	Number of samples	16 samples	4,8,12,16
4	Total processing time	1.5 hrs (Approx.)	16 samples, 30min hypotonic
5	Centrifuge G	370 xg (Approx.)	1400 rpm (r = 17 cm), Max. = 1500rpm
6	Injection pumps	2 (0.1ml/rev)	PBS/Hypotonic x1: 0.1ml - 10ml (+/- 3%) Fixative x1: 0.1ml - 6ml (+/- 3%)
7	Aspiration pumps	4	Twin pump x2
8	Hypotonic treatment temperature	37 ºC	Preheating, Select ON/OFF
9	Centrifuge section temperature	37 ºC	Air Bath, Select ON/OFF
10	Stirrer (Voltex type) Orbital	6 mm	Stirring speed: 0 - 2100rpm
11	Stirring speed select	2	Default: SC1=1500, SC2=2100rpm
12	Protocol Select	6	A, B, C, D, E, F
13	Operating environment	15-30 ºC, 20-60%RH	non-condensing
14	Storage temperature	0 - 40 ºC, 0-95%RH	non-condensing, non-icing
15	Atmospheric Pressure	800 - 1114 hPa	2000 meters or lower
16	Pollution Degree	2	non-conductive levels
17	Input Voltage	100, 120, 230 VAC	Voltage and 50/60Hz are option of the order.
18	Power consumption	800W	
19	Externals size (WxHxD)	850W x 1600H x 700D	Without reagent bottls mount and Safety Filter
20	Weight	250 Kg (Approx.)	
21	Safety Filter (Option)	HEPA + Carbon filter	for EU model
22	Safety Standards	CE-marking	EN61010-1, EN61000-4
23	Protocol Data Reading (Option)	USB	Option

4.2. Outline

US version



EU version

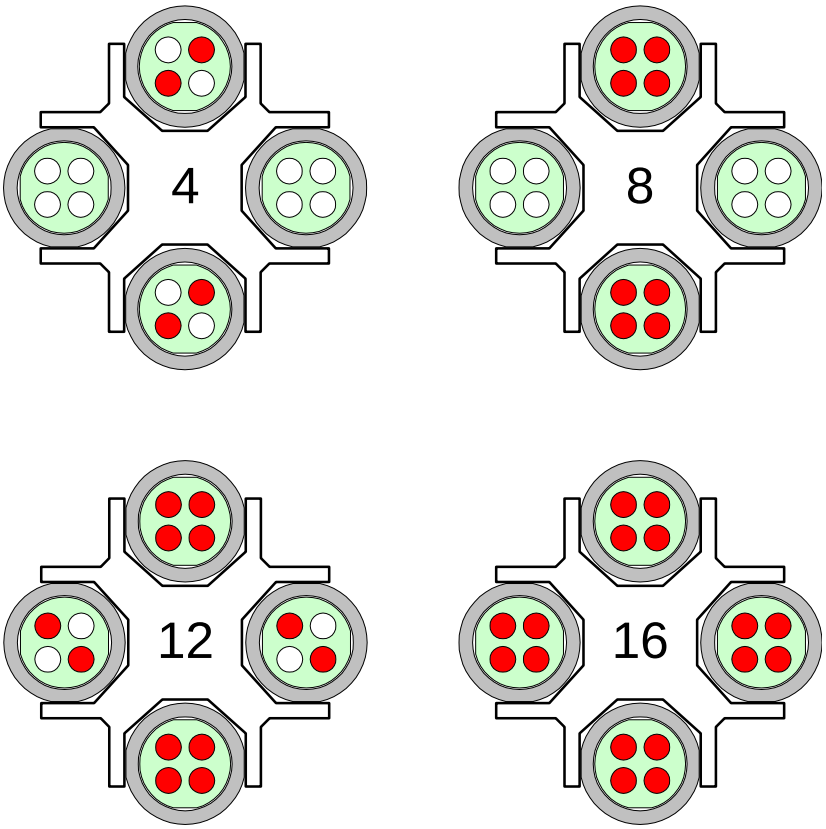


4.3. Combination of samples and dummy samples

The following figure shows the minimum number of sample and dummy tubes respectively in the samples setting mode.

In 4-tube mode, when the minimum number of samples is 1, set 3 dummy tubes with the same amount of distilled water as the sample.

Sample Number	Process Setting	Dummy Tube	Remarks
1	4	3	
4	4	0	
5	8	3	
8	8	0	
9	12	3	
12	12	0	
13	16	3	
16	16	0	



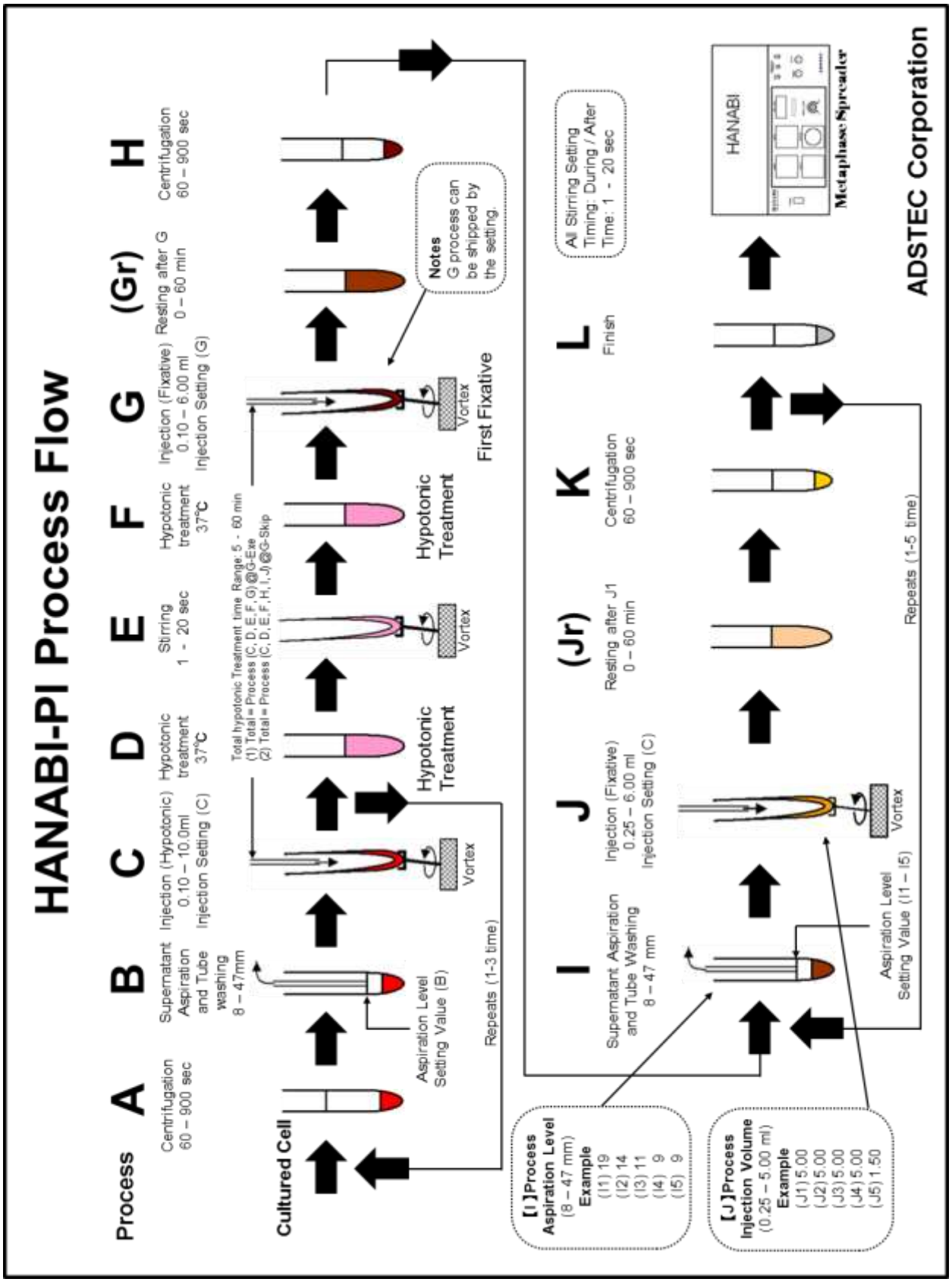
4.4. Range of Setting Items

HANABI-PI Protocol and Ranges

Protocol : A, B, C, D, E, F 2010.07.26

No.	Process	Setting Range			Stirring Conditions			Reagent Select
		Items	Values	Unit	Time	Speed	Mode	
1	A1	Centrifuging time (A1)	0 - 900	sec				
2	A2	Centrifuging time (A2)	0 - 900	sec				
3	A3	Centrifuging time (A3)	0 - 900	sec				
4	B1	Aspiration Level (B1)	8 - 47	mm	0.15ml - 5.0ml			
5	B2	Aspiration Level (B2)	8 - 47	mm	0.15ml - 5.0ml			
6	B3	Aspiration Level (B3)	8 - 47	mm	0.15ml - 5.0ml			
7	C1	PBS/Hypo Injection Vol. (C1)	0.1 - 10.0	ml	1 - 20 sec	SC1 / SC2	A / D	PBS/Hypo
8	C2	PBS/Hypo Injection Vol. (C2)	0.1 - 10.0	ml	1 - 20 sec	SC1 / SC2	A / D	PBS/Hypo
9	C3	PBS/Hypo Injection Vol. (C3)	0.1 - 10.0	ml	1 - 20 sec	SC1 / SC2	A / D	PBS/Hypo
10	ABC	ABC count number	1 - 3	time				
11	D,E,F	Hypotonic total times	5 - 60	min	1 - 20 sec	SC1 / SC2		
12	Gs	Select G process EXE or SKIP	EXE / SKIP					
13	Gi	Fixative Injection Volume (G)	0.1 - 6.0	ml	1 - 20 sec	SC1 / SC2	A / D	
14	Gr	Rest (After first Fixative)	0 - 60	min				
15	H	Centrifuging time (H)	0 - 900	sec				
16	I1	Aspiration Level (I1)	8 - 47	mm	0.15ml - 5.0ml			
17	I2	Aspiration Level (I2)	8 - 47	mm	0.15ml - 5.0ml			
18	I3	Aspiration Level (I3)	8 - 47	mm	0.15ml - 5.0ml			
19	I4	Aspiration Level (I4)	8 - 47	mm	0.15ml - 5.0ml			
20	I5	Aspiration Level (I5)	8 - 47	mm	0.15ml - 5.0ml			
21	J1	Fixative Injection Volume (J1)	0.1 - 6.0	ml	1 - 20 sec	SC1 / SC2	A / D	
23	J2	Fixative Injection Volume (J2)	0.1 - 6.0	ml	1 - 20 sec	SC1 / SC2	A / D	
24	J3	Fixative Injection Volume (J3)	0.1 - 6.0	ml	1 - 20 sec	SC1 / SC2	A / D	
25	J4	Fixative Injection Volume (J4)	0.1 - 6.0	ml	1 - 20 sec	SC1 / SC2	A / D	
26	J5	Fixative Injection Volume (J5)	0.1 - 6.0	ml	1 - 20 sec	SC1 / SC2	A / D	
27	K1	Centrifuging time (K1)	0 - 900	sec				
28	K2	Centrifuging time (K2)	0 - 900	sec				
29	K3	Centrifuging time (K3)	0 - 900	sec				
30	K4	Centrifuging time (K4)	0 - 900	sec				
31	K5	Centrifuging time (K5)	0 - 900	sec				
22	Jr	Rest (After J1)	0 - 60	min				
32	IJK	IJK count number	1 - 5	time				
33		Air Bath Control	ON/OFF/SW					
34		Hypotonic Preheat Control	ON/OFF/SW					

4.5. Operation Process flow chart



5. Appendix

5.1. Reference protocol

This reference protocol is actually used during operation. However, applicability to all samples is not guaranteed. Check applicability of the protocol carefully before operation.

HANABI-PI Protocol Example

2010.07.26

No.	Process	Setting Range			Stirring Conditions			Reagent Select	Setting Values
		Items	Values	Unit	Time	Speed	Mode		
1	A1	Centrifuging time (A1)	0 - 900	sec					300
2	A2	Centrifuging time (A2)	0 - 900	sec					300
3	A3	Centrifuging time (A3)	0 - 900	sec					300
4	B1	Aspiration Level (B1)	8 - 47	mm	1.0ml				19
5	B2	Aspiration Level (B2)	8 - 47	mm	1.0ml				19
6	B3	Aspiration Level (B3)	8 - 47	mm	1.0ml				19
7	C1	PBS/Hypo Injection Vol. (C1)	0.1 - 10.0	ml	5 sec	SC2	After	PBS	5.0
8	C2	PBS/Hypo Injection Vol. (C2)	0.1 - 10.0	ml	5 sec	SC2	After	Hypotonic	5.0
9	C3	PBS/Hypo Injection Vol. (C3)	0.1 - 10.0	ml	5 sec	SC2	After	Hypotonic	5.0
10	ABC	ABC count number	1 - 3	time					3
11	D,E,F	Hypotonic total times	5 - 60	min	5 sec	SC2			35
12	Gs	Select G process EXE or SKIP	EXE / SKIP						EXE
13	Gi	Fixative Injection Volume (G)	0.1 - 6.0	ml	10 sec	SC2	After		0.5
14	Gr	Rest (After first Fixative)	0 - 60	min					0
15	H	Centrifuging time (H)	0 - 900	sec					300
16	I1	Aspiration Level (I1)	8 - 47	mm	1.0ml				19
17	I2	Aspiration Level (I2)	8 - 47	mm	0.5ml				12
18	I3	Aspiration Level (I3)	8 - 47	mm	0.2ml				9
19	I4	Aspiration Level (I4)	8 - 47	mm	0.2ml				9
20	I5	Aspiration Level (I5)	8 - 47	mm	0.2ml				9
21	J1	Fixative Injection Volume (J1)	0.1 - 6.0	ml	10 sec	SC2	After		5.0
23	J2	Fixative Injection Volume (J2)	0.1 - 6.0	ml	5 sec	SC2	After		5.0
24	J3	Fixative Injection Volume (J3)	0.1 - 6.0	ml	5 sec	SC2	After		5.0
25	J4	Fixative Injection Volume (J4)	0.1 - 6.0	ml	5 sec	SC2	After		5.0
26	J5	Fixative Injection Volume (J5)	0.1 - 6.0	ml	5 sec	SC2	After		1.5
27	K1	Centrifuging time (K1)	0 - 900	sec					300
28	K2	Centrifuging time (K2)	0 - 900	sec					150
29	K3	Centrifuging time (K3)	0 - 900	sec					150
30	K4	Centrifuging time (K4)	0 - 900	sec					150
31	K5	Centrifuging time (K5)	0 - 900	sec					150
22	Jr	Rest (After J1)	0 - 60	min					0
32	IJK	IJK count number	1 - 5	time					3
33		Air Bath Control	ON/OFF/SW						SW
34		Hypotonic Preheat Control	ON/OFF/SW						SW

5.2. Operation Check Sheet

Make use of the sheet for daily operations.

HANABI-PI Operation Check Sheet

Date:

Name:

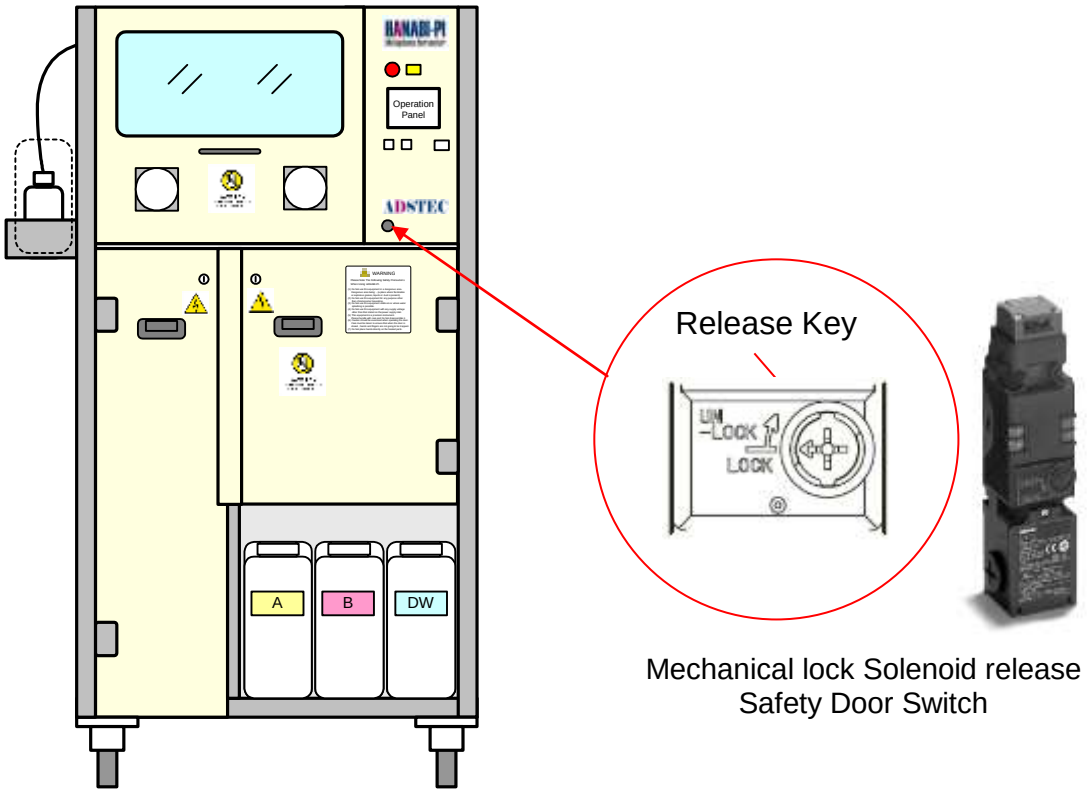
Check	Items	Remarks
Pre-operation check		
	Power ON	
	Check for the solution volume in the distilled water tank	
	Check for the solution volume in the tank (A)	
	Check for the solution volume in the tank (B)	
	Reagent exchange (Distilled water -> Hypotonic solution)	
	Reagent exchange (Distilled water -> PBS)	
	Reagent exchange (Distilled water -> Fixative solution)	
	Pump operation check (Needle washing / Rinse)	
	Protocol setting check	
	Station cover (Check that it's fixed)	
	Protocol ☐A, ☐B, ☐C, ☐D, ☐E, ☐F	
	Number of samples :	
Post-operation check		
	Check for soil in the station	
	Check for soil on the aspiration tube	
	Reagent exchange (Hypotonic solution -> Distilled water)	
	Reagent exchange (PBS -> Distilled water)	
	Reagent exchange (Fixative solution -> Distilled water)	
	Needle washing / Washing with washing water	
	Needle washing / Rinse	
	Pump drying (Reagent exchange / Pump drying)	
	Drainage work of compressor (once a week)	

<Trouble note>

Message contents, etc.

5.3. Release method of safety door lock

An upper door of this equipment has achieved a safe operation with a safety door lock switch.
When power on/off switch OFF and blacking out, this door continues locking.
To release the lock, the rubber cover at the position shown in the photograph (red circle) is removed.
Next, the plus driver turns the release key clockwise 90 degrees.



Mechanical lock Solenoid release
Safety Door Switch

(1) Release Key

The release key is used to unlock the Switch in case of emergency or if the power supply to the Switch stops.

- If the release key setting is changed from LOCK to UNLOCK using an appropriate tool, the lock will be released and the safety door can be opened.
- After setting the release key to UNLOCK to, for example, change the head direction or perform maintenance, be sure to return it to LOCK setting before resuming operation.
- The auxiliary lock must be released by the authorized personnel only.
- Do not impose excessive force on the release key screws. The release key may be damaged and may not operated properly.

5.4. About the management of the key

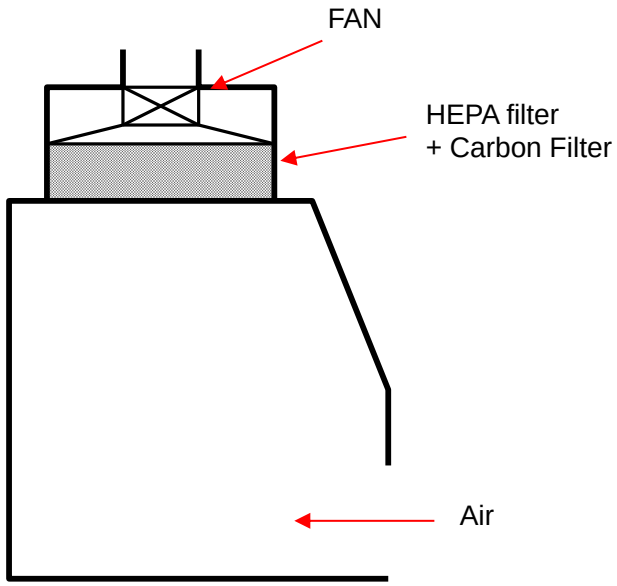
Only the person in charge must operate the release key release of the safety door lock switch.
Moreover, the person in charge or the maintenance professional skill person must open and shut the door with a front, rear key.

5.5. About Microbiological Safety Filter

HANABI-PI has EU model by which safety filter (HEPA) is installed.
EU model is Class-1 cabinet structure that the aerosol doesn't flow out from the equipment at the time of load/unloading the samples.
The cabinet of HANABI-PI is different from a general Microbiological Safety Cabinet. As for the inside, an electromechanical mechanism such as the centrifuge mechanism, the injection parts, and the suction parts is mounted.

Class-I cabinet:

Safety cabinet with a front aperture through which the operator can carry out manipulations inside the cabinet and which is constructed so that the worker is protected and the escape of airborne particulate contamination generated within the cabinet is controlled by means of an inward airflow through the working front aperture and filtration of the exhaust air.



Class-1 cabinet structure

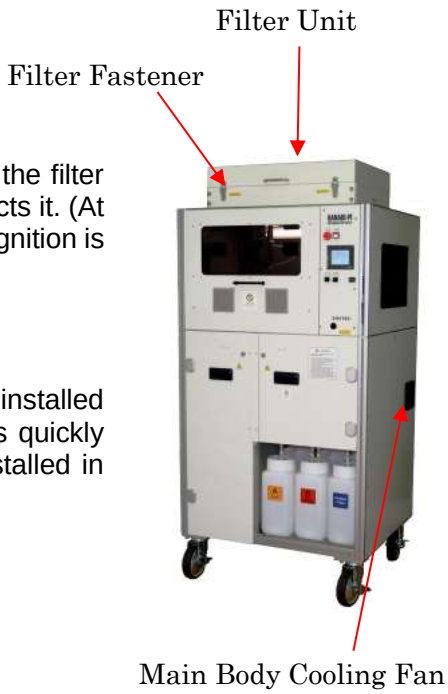
HANABI-PI Cabinet specifications

(1) Front opening area: 0.074m^2
(2) Amount of the air flow: 6.8m^3/min (Fan specification max. airflow)
(3) Velocity of the airflow: >0.6m/s
(4) HEPA filter: 610x400x75
(5) Activated carbon filter: 610x400x50
(6) Noise (rear exhaust part): 60dB

5.6. About the Filter Exchange

Filter unit
The filter exchanges once a year for the standard.

Name: PI Filter Unit
Model: PI-FU101
Power Supply: 24VDC



Replacement procedure:
[Disinfection]
The ethanol for disinfection is sprayed from the inside on the filter while making the filter fan work in the beginning and it disinfects it. (At this time, the evaporating ethanol notes the fire because the ignition is high.)

[Exchange]
The power on/off switch of the equipment is turned off.
The filter fastener is removed. And, it removes in order of installed activated carbon filter and HEPA filter. The detached filter is quickly put in a plastic bag and sealed up. Next, a new filter is installed in order of the HEPA filter and the activated carbon filter.

Specification (type name and part number)
HEPA filter:
HEPA filter (610Hx400Wx75t)
PI-PN0031 (Maintenance parts)
Activated carbon filter:
Activated carbon filter (610Hx400Wx50t)
PI-PN0032 (Maintenance parts)

Filter for main body cooler fan
Fan filter exchanges once a year for the standard.

Replacement procedure:
Fan filter on both sides of the main body is detached, and it exchanges it for a new filter.

Specification (type name and part number)
FL12
PI-PN0033 (Maintenance parts)

Please inquire the question concerning **HANABI-PI** etc.
by the following E-mail, telephone or fax.

Inquiries

ADSTEC Corporation

568-1-1 Innai-cho Funabashi-shi, Chiba, 273-0025 Japan
Tel +81-47-495-9070 Fax +81-47-495-8809

sales@ads-tec.co.jp
sales@adstec-hanabi.com