ISOLATE II
RNA Plant Kit

Product Manual

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ISOLATE II RNA Plant Kit

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1. KIT CONTENTS

COMPONENT	10 Preps	50 Preps
ISOLATE II Filters (violet)	10	50
ISOLATE II RNA Plant Columns (blue) & Collection Tubes	10	50
Lysis Buffer RLY*	10 mL	25 mL
Lysis Buffer RLS*	10 mL	25 mL
Wash Buffer RW1*	15 mL	15 mL
Wash Buffer RW2† (concentrate)	6 mL	12 mL
Membrane Desalting Buffer MEM*	10 mL	25 mL
Reaction Buffer for DNase I RDN	7 mL	7 mL
RNase-free DNase I (lyophilized)	1 vial	1 vial
RNase-free Water	13 mL	13 mL
Collection Tubes (2 mL)	30	150
Collection Tubes (1.5 mL)	10	50
Bench Protocol Sheet	1	1

** Contains a guanidine salt. Not compatible with disinfectants containing bleach or acidic solutions. See safety information in section 4.*

† Before use, add indicated volume of 96-100% ethanol and mark wash buffer bottle label.

2. DESCRIPTION

The ISOLATE II RNA Plant Kit is specially designed for isolation of high-quality total cellular RNA from plant cells, plant tissues or filamentous fungi.

Biological samples are first lysed and homogenized in the presence of guanidinium thiocyanate, a chaotropic salt which immediately deactivates endogenous RNases to ensure purification of intact RNA. After homogenization, ethanol is added to the sample. The sample is then processed through a spin column containing a silica membrane to which the RNA binds. Genomic DNA contamination is removed by an on-column DNase I digestion during the preparation. Any impurities such as salts, metabolites and cellular components are removed by simple washing steps with two different buffers. High quality purified total RNA is then eluted in RNase-free water. The RNA is ready-to-use for a wide variety of applications e.g. qPCR, next generation sequencing, Northern blotting, primer extension, array technology and RNase protection assays.

The ISOLATE II RNA Plant Kit allows convenient processing of multiple samples in <30 min without the need for laborious methods such as CsCl ultracentrifugation or the handling of toxic chemicals e.g. phenol/chloroform.

Please read this manual carefully to familiarize yourself with the ISOLATE II RNA Plant protocol before starting (also available on www.bioline.com). More experienced users can refer to the bench-top protocol for quick referencing during the procedure.

3. STORAGE

Store lyophilized DNase I (RNase-free) at 4°C on arrival (stable for up to 1 year). Reconstituted DNase I working solution is stable for 6 months at -20°C. All other kit components should be stored at room temperature (18–25°C) and are stable for up to 1 year. Storage at lower temperatures may cause precipitation of salts.

4. SAFETY INFORMATION

When working with chemicals, always wear a suitable lab coat, gloves and safety glasses.

Buffers RLY, RW1 and MEM contains guanidinium thiocyanate. This chemical is harmful when in skin contact, inhaled or ingested. Buffer RLS contains guanidine hydrochloride. This chemical is harmful if swallowed and irritating to eyes and skin.

CAUTION: Do not add bleach directly to solutions or sample preparation waste containing guanidine salts. Reactive compounds and toxic gases can form. Clean with a suitable laboratory detergent and water if liquid from these buffers are spilt.

For detailed information, please consult the material data safety sheets (MSDSs) available on our website at www.bioline.com.

5. PRODUCT SPECIFICATIONS

The ISOLATE II RNA Plant Kit is specially designed for the rapid and efficient isolation of extremely pure total RNA from plant cells and tissues or filamentous fungi. The RNA is suited for a wide variety of applications e.g. qPCR and microarrays. The preparation time is approx. 30 min for 6 preps. The isolated RNA is of high-purity (A_{260}/A_{280} ratio: >1.9) and high-integrity (RIN >9) for high-quality samples (see below).

ISOLATE II RNA PLANT SPIN COLUMN SPECIFICATIONS

Max. binding capacity	200 µg RNA
RNA size distribution	>200 nucleotides
A_{260}/A_{280} ratio*	1.9 – 2.1
Typical RIN (RNA integrity number)†	>9
Elution volume	60 µL
Max. amount of starting material Plant tissues Filamentous fungi	 100 mg 100 mg

** Typically, the A_{260}/A_{280} ratio exceeds 1.9, indicating excellent RNA purity*

† Agilent 2100 Bioanalyzer (RNA 6000 assay). RNA integrity is highly dependent on sample quality.

Typical RNA Yield

RNA yield is dependent on sample type, quality and amount. Table 1 shows expected RNA yields from a number of different sources. It is important to use the correct amount of starting material in order to obtain optimal RNA yield and purity.

TABLE 1:
TYPICAL YIELDS OF TOTAL RNA WITH ISOLATE II RNA PLANT KIT

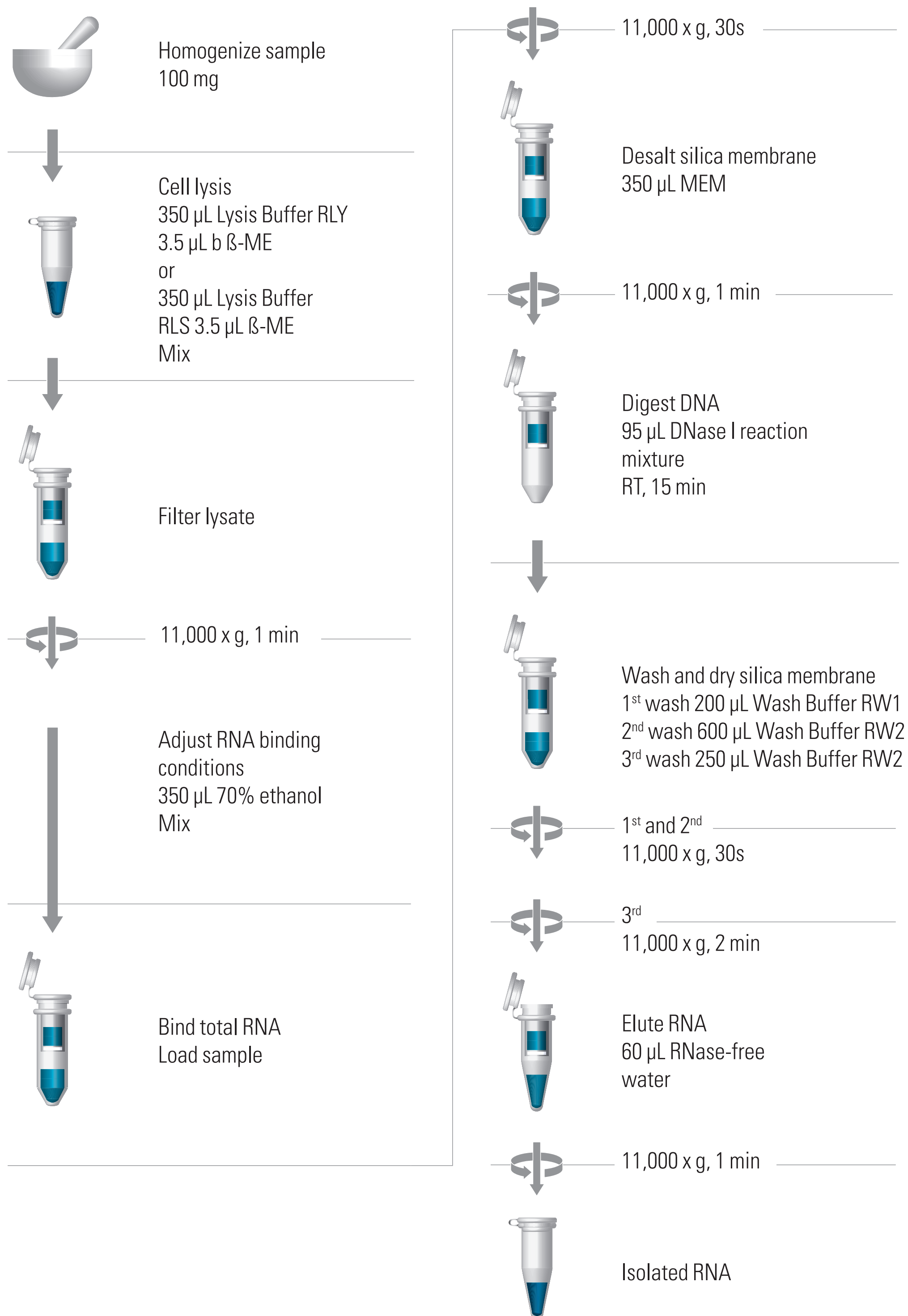
Sample (50 mg material)	Total RNA Yield* (µg)
LEAVES	
Thale cress *(Arabidopsis thaliana)*	15
Maize *(Zea mays)*	18
Common wheat *(Triticum aestivum)*	4
Sugar beet *(Beta vulgaris)*	17
Tomato *(Lycopersicum esculentum)*	10
Tobacco *(Nicotiana tabacum)*	24
MYCELIUM	
Fungus *(Mucor rouxii)*	6

** Yields can vary depending on factors such as species, stage of development and growth conditions.*

The following components are also included in the kit:

- ISOLATE II Filters for homogenization and reduction of lysate viscosity.
- DNase I (RNase-free) for eliminating genomic DNA contamination by on-column digestion or by digestion in solution (for the most sensitive applications).
- Two Lysis Buffers, RLY and RLS containing guanidinium thiocyanate and guanidinium-hydrochloride, respectively.

PLANT TOTAL RNA ISOLATION



6. EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER

When working with chemicals, always wear a suitable lab coat, protective goggles and disposable gloves. Please consult the relevant MSDS from the product supplier for further information.

- ß-mercaptoethanol (ß-ME)* (for Lysis Buffer RLY)
- 70% ethanol† (to adjust RNA binding conditions)
- 96–100% ethanol† (for Wash Buffer RW2)
- Equipment for sample disruption and homogenization (see section 7.2). One or more of the following are required depending on chosen method.
  - Mortar and pestle
  - Rotor-stator homogenizer or bead mills
  - Liquid nitrogen
- Microcentrifuge tubes (1.5 mL)
- Sterile RNase-free tips
- Pipettes
- Microcentrifuge (capable of 11,000 x g)

** Dithiothreitol (DTT) or tris(2-carboxyethyl)phosphine (TCEP) are also suitable reducing agents instead of ß-ME*

† Molecular biology grade ethanol is recommended. Do not use denatured alcohol which contains unwanted additives such as methanol and acetone. Make 70% ethanol up with nuclease-free molecular biology grade water.

7. IMPORTANT NOTES

The ISOLATE II RNA Plant purification procedures can be performed at room temperature. Handle the eluted RNA carefully to avoid contamination by RNases, often found on labware, fingerprints and dust. For optimal RNA stability, keep RNA frozen at -20°C for short-term or -80°C for long-term storage. When working with RNA samples in downstream applications, keep the RNA solution on ice.

See hints and tips on working with RNA at www.bioline.com/isolate

7.1 HANDLING AND STORING STARTING MATERIALS

RNA is not protected against digestion until the sample is flash frozen or disrupted in the presence of RNase inhibiting or denaturing reagents. Samples should be flash frozen in liquid nitrogen immediately, stored at -80°C or processed as soon as possible. Following disruption and homogenization in Lysis Buffer RLY or RLS, samples can be kept at -80°C for up to one year, at 4°C for up to 24 hours or up to several hours at room temperature. Frozen samples are stable for up to 6 months. Frozen samples in Lysis Buffer RLY or RLS should be thawed slowly before starting the isolation of total RNA.

7.2 DISRUPTING AND HOMOGENIZING STARTING MATERIALS

Plant material is often tough, therefore the efficient mechanical disruption and homogenization of starting material is essential. To release all RNA contained in a sample, total disruption of cell walls, plasma membranes and organelles must occur. Incomplete disruption results in reduced RNA yields. For best results, homogenization is required to further reduce lysate viscosity but depends on chosen disruption method. Incomplete homogenization results in inefficient binding of RNA to the silica membrane and therefore reduced RNA yields.

As plant tissue is very robust, the lysis procedure is most effective with well-homogenized, powdered samples. Suitable methods include any type of commercial homogenizers (rotor-stator homogenizer) or bead mills using steel or glass beads. A mortar and pestle is also a commonly used technique to disrupt plant tissues.

Disruption using a mortar and pestle

An RNase-free mortar and pestle can be used in combination with liquid nitrogen to disrupt and lyse frozen plant tissue samples, which are often solid. Grind the frozen tissue into a fine powder and add liquid nitrogen as necessary. It is important to ensure the sample does not thaw during or after grinding. Then transfer the tissue powder into a liquid nitrogen cooled tube and allow the liquid nitrogen to evaporate. Add Lysis Buffer (RLY or RLS with reducing agent) to powdered tissue and mix immediately. Homogenize sample with an ISOLATE II Filter (supplied) or by passing 5-10 times through a nuclease-free 20-gauge (0.9 mm) syringe needle.

Disruption and homogenization using a rotor-stator homogenizer

Rotor-stator type tissue homogenizers can rapidly homogenize, disrupt and emulsify plant tissue samples in the presence of lysis buffer in seconds to minutes. Homogenization time depends on sample size and toughness. Thawing of undisrupted plant tissue should only be performed in presence of lysis buffer to prevent degradation of RNA by RNases. The spinning rotor disrupts and simultaneously homogenizes the sample by turbulence and mechanical shearing. Foaming can be minimized by keeping the rotor tip submerged. Select a suitably sized homogenizer: 5–7 mm diameter rotors can be used for homogenization in microcentrifuge tubes.

Lysis buffer choice

The ISOLATE II RNA Plant Kit provides a choice of two different Lysis Buffers, RLY and RLS containing guanidinium thiocyanate and guanidinium hydrochloride, respectively. In most cases, use of Lysis Buffer RLY is recommended for lysis of most plant material due to its stronger denaturing properties. However, some plant tissues or fungi solidify in Lysis Buffer RLY and the RNA purification cannot proceed. In these cases, Lysis Buffer RLS should be used.

7.3 BUFFER PREPARATION AND PARAMETER

Ensure 70% ethanol and reducing agent (ß-ME, DTT, or TCEP) are available (not supplied).

Prepare the following reagents:

Preparing DNase I (RNase-free) stock solution

Reconstitute lyophilized DNase I in RNase-free water (supplied): 230 µL for the 10 prep kit,

540 µL for the 50 prep kit. Incubate for 1 min at room temperature, then mix by gently swirling.

Important note: *Do not vortex the reconstituted DNase I as the enzyme is particularly sensitive to mechanical denaturation. Dispense into aliquots to avoid excessive freeze-thawing. Do not freeze/thaw aliquots more than three times. Store aliquots at -20°C. The frozen working solution is stable for 6 months.*

Preparing Wash Buffer RW2 with ethanol

Add 96–100% ethanol to Wash Buffer RW2 Concentrate: 24 mL for the 10 prep kit, 48 mL for the 50 prep kit.

Important note: *Mark the label of the bottle to indicate ethanol was added. Store Wash Buffer RW2 at room temperature (18–25°C) for up to one year.*

Elution parameters

Elute RNA using RNase-free water (supplied). The standard elution protocol can be modified for different applications.

- High yield: Perform two successive elution steps with elution volume described in the individual protocol (90–100% recovery rate).
- High yield and high concentration: Elute with the standard elution volume. Then re-apply the eluate onto the column for re-elution.

7.4 ELIMINATING GENOMIC DNA CONTAMINATION

Genomic DNA contamination is removed efficiently by on-column digestion with DNase I (supplied). Residual genomic DNA may be detected in very sensitive applications e.g. probe-based qPCR. A DNase I digest in the eluate can be performed to remove even traces of contaminating DNA (see section 8.2).

8. PROTOCOLS

8.1 PURIFYING TOTAL RNA FROM PLANT TISSUE OR FILAMENTOUS FUNGI

Before you start:

- Ensure Wash Buffer RW2 and DNase I are prepared (see section 7.3)

1 Sample homogenization

Grind sample (up to 100 mg fresh/frozen tissue) under liquid nitrogen to a fine powder using a mortar and pestle. For handling and preparation methods see section 7.1-7.2.

2 Cell lysis

Add 350 µL Lysis Buffer RLY and 3.5 µL ß-ME to a maximum of 100 mg tissue and vortex vigorously. If lysate solidifies when adding Lysis Buffer RLY, use 350 µL Lysis Buffer RLS instead.

Note: *DTT or TCEP may be used in place of ß-ME. Use a final concentration of 10–20 mM DTT or TCEP within the Lysis Buffer RLY or RLS (e.g. add 7–14 µL of a 500 mM DTT or TCEP solution).*

3 Filter lysate

Place ISOLATE II Filter (violet) in a 2 mL Collection Tube (supplied). Load lysate and centrifuge (1 min at 11,000 x g). This step helps reduce viscosity and clears the lysate.

Transfer the filtrate to a new 1.5 mL microcentrifuge tube (not supplied).

Important: *Be careful not to disturb pellet of cell debris in the bottom of the collecting tube, which may be visible after centrifugation.*

In case of visible pellet formation, depending on sample amount and nature, transfer supernatant avoiding any formed pellet to a new 1.5 mL microcentrifuge tube (not supplied).

4 Adjust RNA binding conditions

Discard ISOLATE II Filter and add 350 µL ethanol (70%) to the homogenized lysate.

Mix by pipetting up and down (5 times).

Alternatively, transfer flow-through into a new 1.5 mL microcentrifuge tube (not supplied), add 350 µL ethanol (70%), and mix by vortexing (2 x 5s).

Note: *After addition of ethanol a stringy precipitate may become visible. This will not affect the RNA isolation. Break apart any precipitate by mixing. Do not centrifuge the ethanolic lysate before loading it onto the column to prevent pelleting the precipitate.*

5 Bind RNA

For each preparation place one ISOLATE II RNA Plant Column (blue) in a 2 mL

Collection Tube and load the lysate. Ensure all of the lysate is loaded on the column.

Centrifuge for 30s at 11,000 x g. Place the column in a new 2 mL Collection Tube.

Note: *Maximum loading capacity of ISOLATE II RNA Plant Columns is 750 µL. Repeat procedure if larger volumes are to be processed.*

6 Desalt silica membrane

Add 350 µL Membrane Desalting Buffer (MEM) and centrifuge at 11,000 x g for 1 min to dry the membrane.

Note: *Salt reduces DNase I activity in step 7. If column outlet accidentally touches flow-through, discard flow-through and centrifuge again for 30s at 11,000 x g.*

7 Digest DNA

Prepare DNase I reaction mixture in a sterile 1.5 mL microcentrifuge tube (not supplied): For each isolation, add 10 µL reconstituted DNase I (see section 7.3) to 90 µL Reaction Buffer for DNase I (RDN). Mix by gently flicking the tube.

Apply 95 µL DNase I reaction mixture directly onto the center of the silica membrane of the column. Incubate at room temperature for 15 min.

8 Wash and dry silica membrane

1st Wash

- Add 200 µL Wash Buffer RW1 to the ISOLATE II RNA Plant Column. Centrifuge for 30s at 11,000 x g. Place the column into a new Collection Tube (2 mL).

Wash Buffer RW1 will inactivate the DNase I.

2nd Wash

- Add 600 µL Wash Buffer RW2 to the ISOLATE II RNA Plant Column. Centrifuge for 30s at 11,000 x g. Discard flow-through and place the column back into the Collection Tube.

Note: *Ensure residual buffer from previous steps is washed away with Wash Buffer RW2, especially if lysate has contacted the inner rim of the column during loading of the lysate onto the column. To efficiently wash the inner rim, flush it with Wash Buffer RW2.*

3rd Wash

- Add 250 µL Wash Buffer RW2 to the ISOLATE II RNA Plant Column. Centrifuge for 2 min at 11,000 x g to dry membrane completely. Place the column into a nuclease-free 1.5 mL Collection Tube (supplied).

If for any reason, the liquid level in the Collection Tube during washes 1, 2 or 3 has reached the ISOLATE II RNA Plant Column after centrifugation, discard flow-through and centrifuge again.

9 Elute RNA

Elute the RNA in 60 µL RNase-free water (supplied) and centrifuge at 11,000 x g for 1 min.

If more concentrated RNA is required, perform elution with 40 µL. However, smaller volumes than this may result in reduced RNA yield.

For further alternative elution procedures see section 7.3.

8.2 DNASE I TREATMENT OF PURIFIED RNA IN SOLUTION

The on-column DNase I digestion results in minimal residual DNA, undetectable in most downstream applications. For the most sensitive applications, DNA digestion in solution is recommended to eliminate even traces of contaminating DNA. Stringent RNase control as well as RNA repurification to remove buffer, salts, DNase and digested DNA are needed.

1 Digest DNA (Reaction setup)

Add 6 µL Reaction Buffer for DNase I (RDN) and 0.6 µL DNase I to 60 µL eluted RNA.(Alternatively, premix 100 µL RDN and 10 µL DNase I and add 1/10th volume to one volume of RNA eluate).

Gently swirl tube to mix solution. Gently spin down (approx. 1s at 1,000 x g) to collect all the solution at the bottom of the tube.

2 Sample incubation

Incubate for 10 min at 37°C.

3 RNA repurification

Repurify RNA with a suitable RNA cleanup procedure, e.g. ISOLATE II RNA Micro Clean-Up Kit or by ethanol precipitation.

Ethanol precipitation step

- Add 1/10th volume of sodium acetate (3 M, pH 5.2)
- Add 2.5-3 x volume of 96–100% ethanol to one volume of sample. Mix thoroughly.
- Precipitate for one hour or overnight at -20°C.

Note: *Choose longer incubation times if the sample has a low RNA concentration. Shorter incubation times are sufficient for high RNA concentrations.*

- Centrifuge at maximum speed for 10 min.
- Wash RNA pellet with ice-cold 70% ethanol.
- Dry RNA pellet and resuspend RNA in RNase-free water.

9. TROUBLESHOOTING GUIDE

CLOGGED SPIN COLUMN

POSSIBLE CAUSE	RECOMMENDED SOLUTION
Insufficient disruption or homogenization	Reduce starting material or use larger volume of Lysis Buffer RLY or RLS. Ensure thorough disruption and use ISOLATE II Filters for homogenization of disrupted starting material.
Insufficient centrifugation	Increase centrifugation speed and time.

LOW RNA YIELD OR QUALITY

POSSIBLE CAUSE	RECOMMENDED SOLUTION
Insufficient disruption or homogenization	Reduce starting material or use larger volume of Lysis Buffer RLY or RLS. Ensure thorough disruption and use ISOLATE II Filters for homogenization of disrupted starting material.
Sample material degraded	Store sample material properly. Use fresh material whenever possible; if not, flash-freeze sample in liquid nitrogen. Always keep samples at -80°C. Always add Lysis Buffer before thawing sample. Disrupt samples in liquid nitrogen and ensure tubes are kept chilled.
Incomplete elution	Incubate sample in elution tube with RNase-free water for up to 5 minutes and repeat elution step.
Reagents not properly prepared	Add suitable reducing agent to Lysis Buffer RLY or RLS. Add 96-100% ethanol to Wash Buffer RW2 concentrate. Ethanol is required to create effective binding conditions for RNA to the silica membrane. Add RNase-free water to DNase I.

LOW RNA CONCENTRATION

POSSIBLE CAUSE	RECOMMENDED SOLUTION
High elution volume	Elute RNA with a lower volume.
Inappropriate handling and storing of starting material	Ensure proper handling and storage of samples. Ensure that all steps are followed quickly.

LOW A_{260}/A_{230} RATIO

POSSIBLE CAUSE	RECOMMENDED SOLUTION
Guanidinium thiocyanate carryover	Carefully load lysate to ISOLATE II RNA Plant column, avoid contamination between column and lid. Ensure residual Wash Buffer RW1 is cleaned with Wash Buffer RW2. Apply Wash Buffer RW2 to inner rim of column.

9. TROUBLESHOOTING GUIDE *continued*

DNA CONTAMINATION

POSSIBLE CAUSE	RECOMMENDED SOLUTION
Incorrect lysis	Check protocol has been followed correctly.
Too much starting material	Reduce amount of starting material.
DNase I inactive	Reconstitute lyophilized DNase I and store as recommended.
Oversensitive DNA detection	Use intron spanning primers if possible. Use larger PCR amplicons (DNA detection probability increases with smaller PCR amplicons). Use DNase digestion in solution protocol.

RNA DOES NOT PERFORM WELL IN DOWNSTREAM APPLICATIONS

POSSIBLE CAUSE	RECOMMENDED SOLUTION
Ethanol carryover during elution	Increase centrifugation time for ethanol removal step. Do not allow Wash Buffer RW2 flow through to touch column outlets after second wash.
Salt carryover during elution	Ensure that Wash Buffers are at room temperature. Washing at lower temperatures reduces efficiency of salt removal. Check both solutions for salt precipitates. Resuspend any visible precipitate by gentle warming.
RNA not stored optimally	Always keep eluted RNA on ice to prevent degradation by RNases. Store at -20°C for short term storage or -80°C for long term storage.

A. TECHNICAL SUPPORT

For technical assistance or more information on these products, please email us at tech@bioline.com

B. ORDERING INFORMATION

For technical assistance or more information on these products, please email us at tech@bioline.com

PRODUCT	PACK SIZE	CAT NO.
ISOLATE II RNA Plant Kit	10 Preps	BIO-52076
ISOLATE II RNA Plant Kit	50 Preps	BIO-52077

C. ASSOCIATED PRODUCTS

PRODUCT	PACK SIZE	CAT NO.
ISOLATE II RNA Mini Kit	10 Preps	BIO-52071
ISOLATE II RNA Mini Kit	50 Preps	BIO-52072
ISOLATE II RNA Mini Kit	250 Preps	BIO-52073
ISOLATE II RNA Micro Kit	10 Preps	BIO-52074
ISOLATE II RNA Micro Kit	50 Preps	BIO-52075
RiboSafe RNase Inhibitor	2,500 Units	BIO-65027

D. PRODUCT WARRANTY AND DISCLAIMER

Meridian warrants that its products will conform to the standards stated in its product specification sheets in effect at the time of shipment. Meridian will replace free of charge any product that does not conform to the specifications. This warranty limits Meridian's liability only to the replacement of the product.

NOTES

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Technical Support

For technical assistance or more information on these products, please contact us at mbi.tech@meridianlifescience.com or call us on +49 (0) 3371 60222 03

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