# MyTaq™ Extract-PCR Kit

Shipping: On dry/blue ice

Batch No.: See vial

Catalog numbers:

BIO-21126: 100 reactions

BIO-21127: 500 reactions

**Store at –20°C**

**Storage and stability:**
MyTaq Extract-PCR Kit is shipped on dry/blue ice. On arrival store at -20°C for optimum stability. Repeated freeze/thaw cycles should be avoided. Thaw, mix, and briefly centrifuge each component before use.

**Expiry:**
When stored under the recommended conditions and handled correctly, full activity of the kit is retained until the expiry date on the outer box label.

**Safety precautions:**
Harmful if swallowed. Irritating to eyes, respiratory system and skin. Please refer to the material safety data sheet for further information.

**Quality control specifications:**
MyTaq Extract-PCR Kit and its components are extensively tested for activity, processivity, efficiency, heat activation, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination prior to release.

**Notes:**
For research or further manufacturing use only.

**Trademarks:**
HyperLadder and MyTaq are trademarks of Bioline Reagents Ltd.

## Description

MyTaq™ Extract-PCR Kit offers a convenient, fast and efficient method for the extraction of DNA from a variety of mammalian tissues, particularly from rodent tail or ear samples. The DNA extractions are performed in a single-tube, without the need for multiple washing steps, greatly reducing the risk of sample loss and contamination. The extracted DNA is amplified using the supplied MyTaq HS Red Mix. The advanced formulation of MyTaq HS Red Mix allows fast cycling conditions to be used, greatly reducing the reaction time without compromising PCR specificity or yield.

The specially designed MyTaq HS Red formulation does not interfere with the PCR and enables users to load samples directly onto a gel after the PCR without the need to add loading buffer.

## Components

| | 100 Reactions | 500 Reactions |
|---|---|---|
| Buffer A | 2 x 1 mL | 10 x 1 mL |
| Buffer B | 1 x 1 mL | 5 x 1 mL |
| MyTaq HS Red Mix, 2x | 1 x 1.25 mL | 5 x 1.25 mL |

## Extraction

1. Place between 3 mg and 30 mg tissue sample* into a clean 1.5 mL microfuge tube and add 20 µL buffer A, 10 µL buffer B and 70 µL of water (not supplied). Mix well.
2. Incubate for 5 minutes at 75 °C*, vortexing at least twice during the incubation. Deactivate by heating to 95 °C for 10 minutes.
3. Centrifuge at high speed in a microfuge for one minute to pellet insoluble material and cell debris. Transfer supernatant into a clean 1.5 mL microfuge tube.

* See Important Considerations - Extraction Optimization section if needed.

## PCR Protocol

Dilute supernatant ten-fold in water. For a 25 µL PCR we would recommend using 1 µL of the supernatant as template.

The following protocol is for a standard 25 µL PCR and can be used as a starting point for reaction optimization. Please refer to the Important Considerations and PCR optimization section for further information.

**PCR set-up:**

| Template | 1 to 2 µL |
|---|---|
| Primers (20 µM each) | 0.5 µL |
| MyTaq HS Red Mix, 2x | 12.5 µL |
| Water ($dH_2O$) | up to 25 µL |

**PCR cycling conditions:**

| Step | Temperature | Time | Cycles |
|---|---|---|---|
| Initial denaturation | 95 °C | 3 min | 1 |
| Denaturation | 95 °C | 15 s | 35 |
| Annealing* | User determined | 15 s | |
| Extension* | 72 °C | 20 s | |

* These parameters may require optimization, please refer to the Important Considerations - PCR Optimization section if needed.

## Important Considerations

### Extraction optimization

**Sample size**:
Mouse tail: 1 - 2 mm (3 - 6 mg)
Mouse ear punch: 2 - 4 mm$^2$ (3 - 6 mg)
Other rodent tissue: 3 - 30 mg

**Incubation time:** Extraction incubation time can be extended up to 10 minutes.

**Yield:** Tissue can be diced or crushed into smaller pieces to expose more surface area to the extraction mix resulting in greater yield of extracted DNA.

### PCR optimization

The optimal conditions may vary from reaction to reaction and are dependent on the template/primers used.

**Primers:** Forward and reverse primers are generally used at the final concentration of 0.2 - 0.6 µM each. As a starting point, we recommend using a 0.4 µM final concentration (*i.e.* 10 pmol of each primer per 25 µL reaction volume). Too high a primer concentration can reduce the specificity of priming, resulting in non-specific products.

When designing primers we recommend using primer-design software such as Primer3 (http://frodo.wi.mit.edu/primer3) or visual OMP™ (http://dnasoftware.com) with monovalent and divalent cation concentrations of 10 mM and 3 mM respectively. Primers should have a melting temperature (Tm) of approximately 60 °C.

**Annealing temperature and time:** The optimal annealing temperature is dependent upon the primer sequences and is usually 2-5 °C below the lower Tm of the pair. We recommend starting with a 55 °C annealing temperature and, if necessary, running a temperature gradient to determine the optimal annealing temperature.

**Extension temperature and time:** The extension step should be performed at 72 °C. The extension time is dependent on the length of the amplicon. An extension time of 20 seconds is sufficient for amplicons under 1 kb. For amplification of fragments over 1 kb, we suggest increasing the extension time up to 30 s/kb.

## Troubleshooting Guide

| Problem | Possible Cause | Recommendation |
|---|---|---|
| **No PCR product** | Too much extract in PCR | - Use less tissue sample or cut tissue into smaller pieces.<br>- Use less extract in the PCR, the extract should not be greater than 10% v/v of the total PCR volume. Extracts can be diluted further in water prior to PCR |
| | Inadequate denaturation | - Ensure that tissue extracts are incubated at 95 °C for at least 10 minutes to deactivate extraction mix |
| | Extraction time too short | - Incubate tissue in extraction mix for up to 10 minutes at 75 °C |
| | Missing component in PCR | - Check PCR set-up and volumes used |
| | Defective component in PCR | - Check the integrity and the concentrations of all components as well as the storage conditions. If necessary test each component individually in controlled reactions |
| | PCR cycling conditions not optimal | - Decrease the annealing temperature<br>- Run a temperature gradient to determine the optimal annealing temperature<br>- Increase the extension time, especially if amplifying a long target<br>- Increase the number of cycles |
| | Difficult template | - Increase the denaturation time |
| **Smearing or Non-Specific products** | Excessive cycling | - Decrease the number of cycles |
| | Extension time too long | - Decrease the extension time |
| | Annealing temperature too low | - Increase the annealing temperature |
| | Primer concentration too high | - Decrease primer concentration |
| | Contamination | - Replace each component in order to find the possible source of contamination<br>- Set up the PCR and analyze the PCR product in separate areas |

## Technical Support

If the troubleshooting guide does not solve the problem you are experiencing, please contact your local distributor or our Technical Support with details of reaction set-up, cycling conditions and relevant information.

Email: mbi.tech@meridianlifescience.com

## Associated Products

| Product Name | Pack Size | Cat No |
|---|---|---|
| Agarose | 500 g | BIO-41025 |
| Agarose tablets | 300 g | BIO-41027 |
| HyperLadder™ 1kb | 200 Lanes | BIO-33025 |