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

A Meridian Life Science® Company

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.

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:
Bioline operates under ISO 9001 Management System. 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:
Research use only.

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 1ml	10 x 1ml
Buffer B	1 x 1ml	5 x 1ml
MyTaq HS Red Mix, 2x	1 x 1.25ml	5 x 1.25ml

Extraction

1. Place between 3mg and 30mg tissue sample* into a clean 1.5ml microfuge tube and add 20µl buffer A, 10µl buffer B and 70µl 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 1.5ml microfuge tube.

* See Important Considerations - Extraction Optimization section if needed.

PCR Protocol

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

The following protocol is for a standard 25µl 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 2µl
Primers (20µM each)	0.5µl
MyTaq HS Red Mix, 2x	12.5µl
Water (dH_2O)	up to 25µl

PCR cycling conditions:

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

* 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 - 2mm (3 - 6mg)
Mouse ear punch: 2 - 4mm^2 (3 - 6mg)
Other rodent tissue: 3 - 30mg

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 - 0.6µM each. As a starting point, we recommend using a 0.4µM final concentration (*i.e.* 10pmol of each primer per 25µl 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 10mM and 3mM 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 1kb. For amplification of fragments over 1kb, we suggest increasing the extension time up to 30s/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. - 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 - Run a temperature gradient to determine the optimal annealing temperature - Increase the extension time, especially if amplifying a long target - 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 - 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: tech@bioline.com

Associated Products

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

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