A quantum leap for PCR

MyTaq™ Extract-PCR kit

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- **Easy-to-use - Eliminate complex DNA extraction procedures**
- **Rapid extraction protocol - High-yield, PCR-ready DNA in 15 minutes**
- **Direct gel loading - No need for post-PCR processing**
- **Convenient - Single-tube extraction, minimizes contamination and increases efficiency**
- **Powered by MyTaq™ HS Red Mix - Fast and highly specific amplification**
- **Application validated - Perfect for genotyping**

Fig 1. Overview of the workflow, tissues can be ready for PCR in only 15min.

MyTaq Extract-PCR Kit offers a fast and easy workflow for the extraction and amplification of DNA from a variety of tissue types (Fig 1). Traditional DNA extraction methods are laborious, involving phenol extractions, overnight incubations or column purification steps. Older methods also typically require large volumes of starting material and must be further optimized to aid DNA extraction efficiency, particularly with solid tissue samples. These challenges make traditional DNA extraction methods unsuitable for medium or high-throughput assays that require extracted DNA to be PCR-ready. MyTaq Extract-PCR Kit overcomes all of the challenges associated with existing DNA extraction techniques and consistently delivers high-quality PCR-ready DNA.

MyTaq™ Extract-PCR kit

Simple, single tube extraction

MyTaq Extract-PCR Kit features a novel protease and buffer system that provides fast and efficient lysis in a single tube. The protocol requires minimal user intervention and minimizes sample loss or contamination. MyTaq Extract-PCR Kit delivers high-quality, PCR-ready DNA in as little as 15 minutes in a simple, easy-to-use format (Fig 1).

High performance PCR

MyTaq Extract-PCR Kit is powered by the industry-leading MyTaq HS Red Mix, one of the latest generation of very high performance PCR mastermixes unique to Bioline. MyTaq HS is an antibody-mediated, hot-start enzyme that reduces non-specific amplification and enables room temperature reaction set-up. This unique, optimized enzyme and buffer combination is manufactured to the highest quality control standards and consistently delivers significant improvements in yield, sensitivity and speed over other polymerases.

Direct gel loading and enhanced visualization

MyTaq Extract-PCR Kit buffer system is engineered to allow the direct loading of samples onto agarose gels without the need for further post-PCR processing. The inclusion of an inert red dye improves both handling and visualization properties and increases throughput in the laboratory workflow.

High quality DNA generation

To demonstrate the quality of the DNA produced by MyTaq Extract-PCR Kit, experiments were performed on mouse tail using both MyTaq Extract-PCR Kit and an equivalent kit from Supplier S. Two unique fragments of the same gene were amplified under identical reaction conditions, with the result being MyTaq Extract-PCR Kit consistently generated superior yields in both cases (Fig 2).

Visibly better DNA extraction

Biopsy samples for molecular genotyping techniques using PCR can be problematic owing to the presence of bone, cartilage and blood contaminants. MyTaq Extract-PCR Kit's superior extraction capabilities were demonstrated by subjecting a 2mm snip of mouse tail to a rapid isolation protocol (Fig 3). When used with the same starting material under the same reaction conditions, MyTaq Extract-PCR Kit consistently demonstrated greater sensitivity and higher yield than equivalent kits from other suppliers.

Conclusion

MyTaq Extract PCR Kit offers a rapid, easy-to-use and robust extraction protocol that delivers PCR-ready DNA from a variety of tissue types in as little as 15 minutes. The MyTaq Extract-PCR protocol replaces complex extraction steps with a single-tube extraction procedure, which maximizes DNA yield whilst simultaneously minimizing contamination risks. The simple workflow is further enhanced through the use of MyTaq HS Red Mix for fast, highly specific and sensitive PCR. The inclusion of an inert red dye aids easy visualization and facilitates direct gel loading capabilities, making MyTaq Extract-PCR Kit the method of choice for even the highest throughput genotyping experiments.

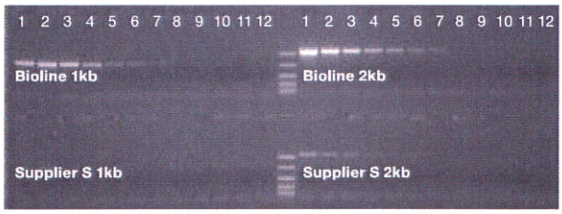

Fig 2. Comparison of amplification of fragments of the mouse cortexin-1 gene (CTXN1).
The MyTaq Extract-PCR Kit and a kit from Supplier S were used to extract and amplify genomic DNA from 3mg pieces of mouse tail according to the manufacturers' instructions. Two-fold serial dilutions (Lanes 1–12) were used for the amplification of a 1kb fragment (A) and a 2kb fragment (B) from the mouse CTXN1 gene. Similar polymerase and isolation conditions were used as specified in the protocol from Supplier S (Lanes 1–12). Marker - EasyLadder I (M).

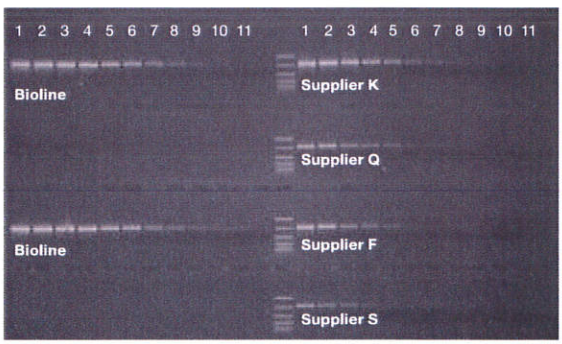

Fig 3. MyTaq Extract-PCR was used to extract and amplify genomic DNA from 3mg pieces of mouse tail.
Genomic DNA was extracted using a 5 minute digestion at 75°C in 100µl of Extraction Buffer, followed by a 10 minute neutralization at 95°C. Cell debris was precipitated by centrifugation and 1µl of the supernatant used for the PCR reactions. After an initial 1 in 30 dilution, serial two fold dilutions of the supernatant were used in 25µl PCR reactions with MyTaq HS Mix and primers for amplification of a 1kb fragment from mouse γ-actin (Lanes 1–11). Marker - EasyLadder I (M).

Ordering Information

PRODUCT	PACK SIZE	CAT NO.
MyTaq Extract-PCR Kit	100 Reactions	BIO-21126
	500 Reactions	BIO-21127

United Kingdom
Tel: +44 (0)20 8830 5300
Germany
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Italy : www.duotech.it
tel 02.33106630 info@duotech.it
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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

BIOLINE
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 allows 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.

Website: www.bioline.com/ email: info@bioline.com

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
PCR water (DNase/RNase free)	10 x 10ml	BIO-38080
HyperLadder™ 1kb	200 Lanes	BIO-33025

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