MyTaq™ Blood-PCR Kit

Shipping: On dry/blue ice

Catalog numbers:

BIO-25054: 250 x 25 µL reactions

Batch No.: See vial

Concentration: 2x

Store at –20°C

Storage and stability:
MyTaq Blood-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:
Please refer to the material safety data sheet for further information.

Quality control specifications:
MyTaq Blood-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™ Blood-PCR Kit is a ready-to-use 2x mix for fast, highly-specific, direct PCR from whole blood samples. MyTaq Blood-PCR Kit is highly optimized for use with whole blood collected with various anticoagulants (EDTA, citrate, heparin) from both human and non-human origins. MyTaq Blood-PCR Kit has been specifically developed to overcome PCR inhibitors typically present in blood samples, to give significantly increased sensitivity and PCR success rates. The advanced formulation of MyTaq Blood-PCR Kit allows fast cycling conditions to be used, without compromising PCR specificity and yield. The speed and high specificity of MyTaq Blood-PCR Kit also makes it highly suitable for end-point multiplex PCR applications.

Components

	250 Reactions
MyTaq Blood-PCR Mix, 2x	5 x 625 µL

Standard MyTaq Blood-PCR Kit Protocol

The following protocol is for a recommended reaction volume of 25 µL and can be used as a starting point for reaction optimization. Reactions can be scaled up where necessary. Please refer to the 'Important Considerations and PCR Optimization' section.

Prepare the following components in a DNase-free reaction tube. Use of DNase-free plasticware and tips is essential.

PCR Set-up:
*The final whole blood concentration in the reaction may require optimization, please refer to the 'Important Considerations and PCR Optimization' section if needed.

Component	Volume
Whole blood*	1 µL (4 % final)*
MyTaq Blood-PCR Mix, 2x	12.5 µL
Primers (25 µM each)	0.5 µL
Water (dH_2O)	up to 25 µL

When running multiple blood samples we recommend creating a master mix containing the 2x MyTaq Blood-PCR Mix, primers and water to reduce pipetting errors. The master mix can then be aliquoted into each reaction tube and the blood samples added.

PCR Cycling Conditions (up to 1 kb):
*These parameters may require optimization, please refer to the 'Important Considerations and PCR Optimization' section if needed.

Step	Temperature	Time	Cycles
Initial denaturation	95 °C	3 min	1
Denaturation	95 °C	15 s	30-40
Annealing*	User determined	15 s	
Extension*	72 °C	45 s	

For PCR of longer amplicons up to 4 kb, we do not recommend exceeding an extension time over 2 minutes.

Important Considerations and PCR Optimization

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

Blood: MyTaq Blood-PCR Kit has been designed for direct amplification from whole blood collected with various anticoagulants (EDTA, citrate and heparin). Although a wide range of blood concentrations can be used (up to 20 %), we recommend a final whole blood concentration of 4 % in the reaction. Using whole blood concentrations over 20 % is not recommended as the pipetting following PCR may be difficult. Blood concentration may require optimization when using blood of non-human origin. With bloods containing nucleated erythrocytes such as avian blood we suggest reducing the final whole blood concentration.

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 0.5 µM final concentration. 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.

Initial denaturation: The initial denaturation step is required to activate the enzyme and fully melt the template. We recommend 3 minutes of initial denaturation at 95 °C.

Denaturation: Our protocol recommends a 15 s cycling denaturation step at 95 °C, which is also suited to GC-rich templates (>55 %).

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 depends on the length of the amplicon and the complexity of the template. An extension time of 45 s is sufficient for amplicons up to 1 kb. For amplification of longer fragments up to 4 kb, longer extension times are recommended. We do not recommend extension times over 2 minutes.

Multiplex PCR Protocol

MyTaq Blood-PCR Kit is suitable for multiplex PCR. Adjustment of the cycling conditions on the thermocycler may be required. As a starting point we recommend using the following conditions:

Recommended standard cycling conditions for multiplex PCR

Step	Temperature	Time	Cycles
Initial denaturation	95 °C	3 min	1
Denaturation	95 °C	30 s	25-40*
Annealing/Extension*	User determined	3 min*	

* These parameters may require optimization, please refer to the Important Considerations and PCR Optimization section if needed. The annealing/extension time will increase as the number of reactions included in the multiplex, and the length of the amplicons, increases.

Multiplexing: When performing multiplex PCR, the recommended 2-step cycling protocol can be further optimized as follows:

Annealing/extension temperature: We highly recommend performing an initial temperature gradient to determine the optimal annealing temperature required for the primer sets used.

Annealing/extension time: A 3 minute annealing/extension step is normally sufficient in most cases. However, depending on the degree of multiplexing to be performed, this step may require longer annealing/ extension times.

Cycling number: We recommend starting with 30 cycles and to optimize this parameter if necessary. An excess of cycles may generate diffuse bands, too few may result in weak or no amplification.

Troubleshooting Guide

Problem	Possible Cause	Recommendation
No or weak amplification	Missing component	- Check the reaction set-up and volumes used
	Defective component	- Check the aspect and the concentrations of all components as well as the storage conditions. If necessary test each component individually in controlled reactions
	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
	Primer purity or design not ideal	- Check the purity and concentration of primers. Re-design new primers if required.
	Inhibition by sample	- Use a smaller amount of blood as template or diluted sample with nuclease-free PCR water. Try an initial two-fold dilution series
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 and then run a temperature gradient PCR to determine optimal annealing temperature
	Primer concentration too high	- Decrease primer concentration
	Primer purity or design not ideal	- Check the purity and concentration of primers. Re-design new primers if required
	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 difficulty 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
HyperLadder™ 1kb	200 Lanes	BIO-33025
ISOLATE II Blood DNA Kit	50 Preps	BIO-52063

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