MyTaq™ Red DNA Polymerase

Shipping: On Dry / Blue ice

Catalog numbers:

BIO-21108 : 500 Units

Batch No.: See vial

BIO-21109 : 2500 Units

Concentration: 5 U/μL

BIO-21110 : 5000 Units

Store at –20 °C

meridian BIOSCIENCE™

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

Unit definition:
One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTPs into acid-insoluble form in 30 minutes at 72 °C.

Notes:
For research or further manufacturing use only.

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

Description

MyTaq™ Red DNA Polymerase is a high performance PCR product that exhibits more robust amplification than other commonly used polymerases. MyTaq Red DNA Polymerase delivers very high yield over a wide range of PCR templates and making it the ideal choice for most routine assays. This new enzyme preparation is supplied with MyTaq red reaction buffer system, a proprietary formulation that saves time and delivers superior results, containing dNTPs, $MgCl_2$ and enhancers at optimal concentrations which eliminates the need for optimization.

The specially designed MyTaq 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.

MyTaq Red only requires the addition of template, primers and water, thus reducing the risk of pipetting errors and contamination, as well as shortening the set-up time.

Components

	500 Units	2500 Units	5000 Units
MyTaq Red DNA Polymerase	1 x 100 μL	2 x 250 μL	4 x 250 μL
5x MyTaq Red Reaction Buffer	4 x 1 mL	14 x 1.5 mL	9 x 5 mL

Standard MyTaq Red Protocol

The following protocol is for a standard 50 μL reaction and can be used as a starting point for reaction optimization.

PCR reaction set-up:

All reactions must be set-up on ice.

5x MyTaq Red Reaction Buffer	10 μL
Template	as required
Primers 20 μM each	1 μL
MyTaq Red DNA Polymerase	1 μL
Water (ddH_2O)	up to 50 μL

PCR cycling conditions:

We suggest these conditions in the first instance:

Step	Temperature	Time	Cycles
Initial denaturation	95 °C	1 min	1
Denaturation	95 °C	15 s	25-35
Annealing*	User determined	15 s	
Extension*	72 °C	10 s	

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

Important Considerations and PCR Optimization

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

5x MyTaq Red Buffer: The 5x MyTaq Red Reaction Buffer comprises 5 mM dNTPs, 15 mM $MgCl_2$, stabilizers and enhancers. The concentration of each component has been extensively optimized, reducing the need for further optimization. Additional PCR enhancers such as Betaine etc. are not recommended.

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 0.4 μM as a final concentration (i.e. 20 pmol of each primer per 50 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

Template: The amount of template in the reaction depends mainly on the type of DNA used. For templates with low structural complexity, such as plasmid DNA, we recommend using 50 pg-10 ng DNA per 50 μL reaction volume. For eukaryotic genomic DNA, we recommend a starting amount of 200 ng DNA per 50 μL reaction, this can be varied between 5 ng-500 ng. It is important to avoid using template resuspended in EDTA-containing solutions (*e.g.* TE buffer) since EDTA chelates free Mg^{2+}.

Initial denaturation: An initial denaturation step of 1 min at 95 °C is recommended for non-complex templates such as plasmid DNA or cDNA. For more complex templates such as eukaryotic genomic DNA, longer initial denaturation times of up to 3 mins are required in order to facilitate complete melting of the DNA.

Denaturation: Our protocol recommends a 15 s cycling denaturation step at 95 °C which is also suited to GC-rich templates, however for low GC content (40-45 %) templates, the denaturation time can be decreased to 5 s.

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 running a temperature gradient to determine the optimal annealing temperature, alternatively 55 °C can be used as a starting point. Depending on the reaction the annealing time can also be reduced to 5 s.

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, to run a temperature gradient to determine the optimal annealing temperature. Depending on the reaction the annealing time can also be reduced to 5 s.

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. With low-complexity template such as plasmid DNA, an extension time of 10 s is sufficient for amplicons of under 1 kb or up to 5 kb. For amplification of fragments over 1 kb from a high complexity template, such as eukaryotic genomic DNA, longer extension times are recommended. In order to find the fastest optimal condition, we suggest incrementing the extension time successively up to 30 s/kb.

Troubleshooting Guide

Problem	Possible Cause	Recommendation
No PCR product	Missing component	- Check 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
	Enzyme concentration too low	- Increase enzyme quantity to up to 5U/50 μL reaction
	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
	Extension during set-up	- Make sure all reactions are set-up on ice. Run reaction as quickly as possible
	Contamination	- Replace each component in order to find the possible source of contamination - Setup the PCR and analyze the PCR product in separated 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 data.

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

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