



DATA SHEET

Storage and stability:

The MyTaq Red is shipped on Dry/Blue Ice and can be stored for up to 12 months at -20°C, or up to 2 weeks at +4°C. Repeated freeze/thaw cycles should be avoided.

Safety precautions:

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

Unit definition:

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

Notes:

Research Use Only.

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 from Bioline is supplied with MyTaq red reaction buffer system, a proprietary formulation that saves time and delivers superior results, containing dNTPs, MgCl₂ and enhancers at optimal concentrations which eliminates the need for optimization.

The specially designed MyTaq Red formulation does not interfere with the PCR reaction 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 DNA Polymerase	1 x 100µl	2 x 250µl	4 x 250µl
5x MyTaq Red Reaction Buffer	4 x 1ml	14 x 1.5ml	9 x 5ml

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 5mM dNTPs, 15mM MgCl₂, stabilizers and enhancers. The concentration of each component has been extensively optimized, reducing the need for further optimization. Additional PCR enhancers such as HiSpec, PolyMate or Betaine etc. are not recommended.

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 0.4µM as a final concentration (i.e. 20pmol of each primer per 50µl 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 (T_m) 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 50pg-10ng DNA per 50µl reaction volume. For eukaryotic genomic DNA, we recommend a starting amount of 200ng DNA per 50µl reaction, this can be varied between 5ng-500ng. It is important to avoid using template re-suspended in EDTA-containing solutions (e.g. TE buffer) since EDTA chelates free Mg²⁺.

Initial Denaturation: An initial denaturation step of 1min 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 3mins are required in order to facilitate complete melting of the DNA.

Denaturation: Our protocol recommends a 15s 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 5s.

Annealing temperature and time: The optimal annealing temperature is dependent upon the primer sequences and is usually 2-5°C below the lower T_m 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 5s.

Standard MyTaq Red Protocol

The following protocol is for a standard 50µl 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	10µl
Template	as required
Primers 20µM each	1µl
MyTaq Red DNA Polymerase	0.25 - 1µl
Water (ddH ₂ O)	up to 50µl

PCR cycling conditions:

We suggest these conditions in the first instance:

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

Annealing temperature and time: The optimal annealing temperature is dependent upon the primer sequences and is usually 2-5°C below the lower T_m 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 5s.

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 10s is sufficient for amplicons of under 1kb or up to 5kb. For amplification of fragments over 1kb 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 30s/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/50µl 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 - Set-up the PCR reaction and analyze the PCR product in separated areas.

Associated Products

Product Name	Pack Size	Cat. No.
Agarose	500g	BIO-41025
Agarose tablets	300g	BIO-41027
PCR water (DNase/RNase free)	10x 10ml	BIO-38080
HyperLadder I	200 Lanes	BIO-33025

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium