DATA SHEET

Storage and stability:
The MyTaq HS 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 10nmoles of dNTPs into acid-insoluble form in 30 minutes at 72°C.

Notes:
Research Use Only.

Description

MyTaq™ HS DNA Polymerase is a high performance PCR product powered by antibody mediated hot-start, specifically designed for fast, highly-specific, hot-start PCR. The product also has the added convenience of room temperature reaction assembly, without non-specific amplification and primer-dimer formation. This new hot-start enzyme preparation from Bioline is supplied with 5x MyTaq Reaction Buffer, a proprietary formulation containing dNTPs, $MgCl_2$ and enhancers at optimal concentrations, removing the need for optimization and providing superior amplification.

Components

	250 Units	1000 Units	2500 Units
MyTaq HS DNA Polymerase	1 x 50µl	1 x 200µl	2 x 250µl
5x MyTaq Reaction Buffer	2 x 1ml	8 x 1ml	14 x 1.5ml

Standard MyTaq HS Protocol

The following protocol is for a standard 50µl reaction and can be used as a starting point for reaction optimization. Please refer to the Important Considerations and PCR Optimization section.

PCR reaction set-up:

5x MyTaq Reaction Buffer	10µl
Template	as required
Primers 20µM each	1µl
MyTaq HS DNA Polymerase	0.25 - 1µl
Water (ddH_2O)	up to 50µl

PCR cycling conditions:

Step	Temperature	Time	Cycles
Initial denaturation	95°C	1min	1
Denaturation	95°C	15s	25-35
Annealing	55°C	15s	
Extension	72°C	10s	

Colony PCR:

MyTaq HS can be used for amplification of plasmid DNA directly from liquid cultures or from colonies on agar plates:

- From liquid culture: up to 8µl of the overnight culture can be directly added to the final reaction mix.
- From colonies: we recommend using a sterile tip to stab the colony and resuspend it directly in the 50µl reaction mix.

Recommended cycling conditions for colony PCR of fragment up to 1kb.

Step	Temperature	Time	Cycles
Initial denaturation	95°C	1min	1
Denaturation	95°C	15s	25-35
Annealing	55°C	15s	
Extension	72°C	10s	

* These steps may require optimization, please refer to the PCR optimization section if needed.

Multiplex PCR:

MyTaq HS is suitable for multiplex PCR, but adjustment of the annealing temperature and extension time may be required.

- Annealing temperature: We suggest using 55°C as a starting annealing temperature. If further optimization is required we recommend using a temperature gradient to determine the optimal annealing temperature needed for the multiplex PCR.
- Extension time: since multiplex PCR generally requires a longer extension step, we suggest starting with a minimum of 90s and increasing it if required.

Recommended cycling conditions for multiplex PCR of fragment up to 1kb.

Step	Temperature	Time	Cycles
Initial denaturation	95°C	1min	1
Denaturation	95°C	15s	25-35
Annealing	55°C	15s	
Extension	72°C	90s	

* These steps may require optimization, please refer to the 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 Reaction Buffer: The 5x MyTaq Reaction Buffer comprises of 5mM dNTPs, 15mM $MgCl_2$, stabilizers and enhancers. The concentration of each component has been extensively optimized, reducing the need for further optimization. Additional PCR enhancers such as 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 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 cations concentrations of 10mM and 3mM 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 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 Mg^{2+}.

Initial Denaturation: The initial denaturation step is required to activate the enzyme and fully melt the template. We recommend 1 minute of initial denaturation at 95°C, however for more complex templates such as eukaryotic genomic DNA, longer initial denaturation times of up to 3 minutes might be required.

Denaturation: Our protocol recommends a 15s cycling denaturation step at 95°C which is also suited to GC-rich templates. For low GC content (40-45%) amplicons, the denaturation step can be decreased down to 5s, however for templates containing high GC content of >55%, a longer denaturation time of 15s is recommended.

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 down 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. For low complexity templates such as plasmid DNA or cDNA, an extension time of 10s is sufficient for amplicons of under 1kb or up to 5kb.

For amplification of fragments over 1kb from high complexity template, longer extension times are recommended. In order to find the fastest optimal condition, we suggest incrementing successively the extension time 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
	Contamination	- Replace each components 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

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