GENTAUR

DATA SHEET

Storage and stability:
The MyTaq HS Mix 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.

Quality control:
Bioline operates under ISO 9001 Management System. MyTaq HS Mix 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™ HS Mix is a ready-to-use 2x mix for fast, highly-specific, hot-start PCR. MyTaq HS Mix is powered by antibody mediated hot-start and does not possess polymerase activity during the reaction set-up, thus reducing non-specific amplification. The advanced formulation of MyTaq HS Mix allows fast cycling conditions to be used, greatly reducing the reaction time without compromising PCR specificity and yield. Thanks to its speed and high specificity MyTaq HS Mix is also highly suitable for end point multiplex PCR. MyTaq HS Mix contains all the reagents (including stabilizers) necessary for trouble-free PCR set up. The product is supplied conveniently all in one tube to reduce the number of pipetting steps and to facilitate increased efficiency, throughput and reproducibility.

Components

	200 Reactions	1000 Reactions
MyTaq HS Mix, 2x	4 x 1.25ml	20 x 1.25ml

Standard MyTaq HS Mix 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 set-up:

Template	200ng
Primers (20µM each)	1µl
MyTaq HS Mix, 2x	25µl
Water (dH_2O)	up to 50µl

PCR cycling conditions:

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

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

Colony PCR Protocol

MyTaq HS Mix 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*	User determined	15s	
Extension*	72°C	10s	

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

Multiplex PCR Protocol

MyTaq HS Mix is suitable for multiplex PCR; adjustment of the cycling conditions 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	2min	1
Denaturation	95°C	30s	25*
Annealing/Extension*	User determined	4min*	

* 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 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.* 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 (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 resuspended 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 may be required.

Denaturation: Our protocol recommends a 15s cycling denaturation step at 95°C, which is also suited to GC-rich templates (>55%). For low GC content amplicons (40-45%), the denaturation step 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 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. 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. An extension time of 10s is sufficient for amplicons under 1kb or up to 5kb for low complexity template such as plasmid DNA. For amplification of fragments over 1kb from high complexity template, such as eukaryotic genomic DNA, longer extension times are recommended. In order to find the fastest optimal condition, we suggest increasing the extension time up to 30s/kb.

Multiplexing: When doing multiplex PCR the recommended 2-step cycling protocol may be optimized as follows:

- Annealing/extension temperature: we highly recommend initially using a temperature gradient to determine the optimal annealing temperature needed for the primer set used.
- Annealing/extension time: in most cases a 4min annealing/extension step is largely sufficient. However in order to reduce the overall cycling time this step can be reduced down to 1min, especially in the case of a lower number of multiplex amplicons.
- Cycling number: we recommend starting with 25 cycles and if necessary, optimizing this parameter. An excess of cycles may generate diffuse bands, too few may result in weak or no amplification.

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
	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.

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-37080
HyperLadder™ I	200 Lanes	BIO-33025
SureClean Plus	1 x 5ml	BIO-37047

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium