DATA SHEET

Storage and stability:
The MyTaq 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 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™ Mix is a ready-to-use 2x mix developed for fast and highly-specific PCR. The advanced formulation of MyTaq Mix exhibits more robust amplification than other commonly used polymerases, delivering a very high yield over a wide range of PCR templates, and making it the ideal choice for most routine assays. MyTaq Mix contains all the reagents (including stabilizers) necessary for trouble-free PCR reaction set up. The product is conveniently supplied all in one tube, reducing the number of pipetting steps and facilitating increased efficiency, throughput and reproducibility.

Components

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

Standard MyTaq Mix 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.

Template	200ng
Primers (20µM each)	1µl
MyTaq Mix, 2x	25µl
Water (ddH_2O)	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	

Important considerations and PCR optimization

The optimal conditions will 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 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 (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: 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 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 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 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
	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-37080
HyperLadder™ I	200 Lanes	BIO-33025
SureClean Plus	1 x 5ml	BIO-37047

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