

# MyFi™ Mix

Shipping: On Dry/Blue Ice Catalog numbers

Exp. Date: See vial BIO-25049: 100 x 50µl reactions: 2 x 1.25ml

Batch No.: See vial BIO-25050: 500 x 50µl reactions: 10 x 1.25ml

Concentration: 2x

Store at -20°C



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## Storage and stability:

The MyFi 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. MyFi 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

MyFi Mix is a newly developed ready-to-use 2x mix, specifically designed for TA cloning, that offers 3.5x higher fidelity than native *Taq*. MyFi Mix can amplify genomic DNA up to 10kb and owing to its antibody-based hot-start property, MyFi Mix has the added convenience of room temperature reaction assembly, avoiding unwanted non-specific amplification including primer-dimer formation. This new hot-start enzyme preparation from Bioline is supplied as a 2x formulation containing MyFi DNA Polymerase, dNTPs, MgCl<sub>2</sub> and enhancers at optimal concentrations, removing the need for optimization and delivering superior amplification.

## Components

	100 Reactions	500 Reactions
MyFi Mix, 2x	2 x 1.25ml	10 x 1.25ml

## Standard MyFi 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 reaction set-up:

Template	200ng
Primers (20µM each)	1µl
MyFi Mix, 2x	25µl
Water (ddH <sub>2</sub> O)	up to 50µl

### PCR cycling conditions:

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

\* Temperature is primer dependent

\*\* Up to 1kb (for greater than 1kb please refer to Important Considerations and PCR Optimization section)

## 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 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 (T<sub>m</sub>) 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<sup>2+</sup>.

**Initial Denaturation:** The initial denaturation step is required to activate the enzyme and fully melt the template. For most PCR, 1 minute at 95°C is sufficient to melt the DNA template, however we recommend up to 3min for complex templates such as eukaryotic genomic DNA.

**Denaturation:** We recommend a 15s cycling denaturation step at 95°C, which is also suited to GC-rich templates. Increasing this step to 30s may improve problematic reactions.

**Annealing temperature and time:** The optimal annealing temperature is dependent upon the primer sequences and is usually 2-5°C below the lower T<sub>m</sub> 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. Although a 15s annealing step will be sufficient in most cases, increasing it up to 45s may improve problematic reactions.

**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 15s is generally sufficient for amplicons up to 1kb. For amplification of fragments over 1kb, longer extension times are recommended. In order to find the fastest optimal condition, the extension time may be increased up to 45s/kb.

## Troubleshooting Guide

Problem	Possible Cause	Recommendation
<b>No PCR product</b>	Missing component	- Check reaction set-up
	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 - Redesign primers
	Cycling conditions not optimal	- 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 initial denaturation time up to 3min
<b>Smearing or Non-Specific products</b>	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
	Suboptimal primer design	- Check that the primers are working in a control reaction - Check primer design
	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
<b>Low Yield</b>	Insufficient cycling	- Increase the number of cycles
	Extension time too short	- Increase the extension time up to 45s/kb
	Not enough template	- increase template concentration

## 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 setup, cycling conditions and relevant data.

Email: [tech@bioline.com](mailto:tech@bioline.com)

## 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
SureClean Plus	1 x 5ml	BIO-37047

## TRADEMARK AND LICENSING INFORMATION

- 1). Notice to Purchaser: Licensed under U.S. patent numbers 5,338,671 and 5,587,287 and corresponding patents in other countries
- 2). MyFi and HyperLadder are Trademarks of Bioline Ltd.

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