

# MyFi™ Mix

Shipping: On Dry/Blue Ice Catalog numbers

BIO-25049: 100 x 50 µL reactions: 2 x 1.25 mL

Batch No.: See vial

BIO-25050: 500 x 50 µL reactions: 10 x 1.25 mL

Concentration: 2x

Store at -20°C



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## Storage and stability:

The MyFi Mix is shipped on dry/blue ice. On arrival store at -20 °C for optimum stability. Repeated freeze/thaw cycles should be avoided.

## Expiry:

When stored under the recommended conditions and handled correctly, full activity of the kit is retained until the expiry date on the outer box label.

## Safety precautions:

Please refer to the material safety data sheet for further information.

## Quality control specifications:

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.

## Note:

For 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 10 kb 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 1.25 mL	10 x 1.25 mL

## Standard MyFi Mix Protocol

The following protocol is for a standard 50 µL 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	200 ng
Primers (20 µM each)	1 µL
MyFi Mix, 2x	25 µL
Water (ddH <sub>2</sub> O)	up to 50 µL

### PCR cycling conditions:

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

\* Temperature is primer dependent

\*\* Up to 1 kb (for greater than 1 kb 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 0.2-0.6 µM each. As a starting point, we recommend using a 0.4 µM final concentration (i.e. 20 pmol of each primer per 50 µL 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 10 mM and 3 mM 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 50 pg-10 ng DNA per 50 µL reaction volume. For eukaryotic genomic DNA, we recommend a starting amount of 200 ng DNA per 50 µL reaction; this can be varied between 5 ng-500 ng. 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 3 min for complex templates such as eukaryotic genomic DNA.

**Denaturation:** We recommend a 15 s cycling denaturation step at 95 °C, which is also suited to GC-rich templates. Increasing this step to 30 s 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 15 s annealing step will be sufficient in most cases, increasing it up to 45 s 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 15 s is generally sufficient for amplicons up to 1 kb. For amplification of fragments over 1 kb, longer extension times are recommended. In order to find the fastest optimal condition, the extension time may be increased up to 45 s/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 3 min
<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 45 s/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	500 g	BIO-41025
Agarose tablets	300 g	BIO-41027
HyperLadder™ 1kb	200 Lanes	BIO-33025
SureClean Plus	1 x 5 mL	BIO-37047

## TRADEMARKS

1. MyFi and HyperLadder are Trademarks of Bioline Ltd.

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