

MyFi™ DNA Polymerase

Shipping: On Dry/Blue Ice Catalog numbers
BIO-21117 : 250 Units
Batch No.: See vial BIO-21118 : 500 Units
Concentration: 2U/ μ l BIO-21119 : 2500 Units

Store at -20°C



A Meridian Life Science® Company

Storage and stability:

The MyFi 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.

Unit definition:

One unit is defined as the amount of enzyme that incorporates 10nmols of dNTPs into acid-insoluble form in 30 minutes at 72°C.

Quality control specifications:

MyFi 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™ DNA Polymerase is a newly developed PCR enzyme specifically designed for TA cloning that offers 3.5x higher fidelity than native *Taq*. MyFi DNA Polymerase can amplify genomic DNA up to 10kb and owing to its antibody-based hot-start property, 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 with 5x MyFi Reaction Buffer, a proprietary formulation containing dNTPs, MgCl₂ and enhancers at optimal concentrations, removing the need for optimization and delivering superior amplification.

Components

	250 Units	500 Units	2500 Units
MyFi DNA Polymerase	1 x 125 μ l	1 x 250 μ l	2 x 625 μ l
5x MyFi Reaction Buffer	1 x 625 μ l	1 x 1.25ml	5 x 1.25ml

Standard MyFi Protocol

The following protocol is for a standard 25 μ l 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 MyFi Reaction Buffer	5 μ l
Template	as required
Primers 20 μ M each	0.5 μ l
MyFi DNA Polymerase	1 μ l*
Water (dH ₂ O)	up to 25 μ l

* We do not recommend using less than 1 μ l in a 25 μ l reaction

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.

5x MyFi Reaction Buffer: The 5x MyFi Reaction Buffer contains 1mM dNTPs, 3mM MgCl₂ (final concentration) and enhancers. The concentration of each component has been extensively optimized, reducing the need for further optimization. Additional dNTPs and PCR enhancers such as HiSpec, PolyMate or DMSO etc. are not recommended and they could lead to PCR failure.

Primers: Forward and reverse primers are generally used at the final concentration of 0.2-0.6 μ M each. As a starting point, we recommend using a 0.4 μ M final concentration (i.e. 20pmol of each primer per 50 μ l 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_m) 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 50 μ l reaction volume. For eukaryotic genomic DNA, we recommend a starting amount of 200ng DNA per 50 μ l 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 Mg²⁺.

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 suitable for GC-rich templates. Increasing this step up 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_m 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 sufficient for amplicons under 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
No PCR product	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
Smearing or Non-Specific products	Excessive cycling	- Decrease the number of cycles
	DNA polymerase concentration too high	- Decrease the amount of DNA polymerase per reaction
	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
Low Yield	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
	DNA polymerase concentration too low	- Increase the amount of DNA polymerase per reaction

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

Associated Products

Product Name	Pack Size	Cat No
Agarose	500g	BIO-41025
Agarose tablets	300g	BIO-41027
HyperLadder™ 1kb	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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