MyFi™ DNA Polymerase

Shipping: On Dry/Blue Ice

Catalog numbers

BIO-21117 : 250 Units

Batch No.: See vial

BIO-21118 : 500 Units

Concentration: 2 U/μL

BIO-21119 : 2500 Units

Store at –20°C

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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 10nmoles 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 10 kb 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, $MgCl_2$ 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 μL	1 x 250 μL	2 x 625 μL
5x MyFi Reaction Buffer	1 x 625 μL	1 x 1.25 mL	5 x 1.25 mL

Standard MyFi Protocol

The following protocol is for a standard 25 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:

5x MyFi Reaction Buffer	5 μL
Template	as required
Primers 20 μM each	0.5 μL
MyFi DNA Polymerase	1 μL*
Water (dH_2O)	up to 25 μL

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

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.

5x MyFi Reaction Buffer: The 5x MyFi Reaction Buffer contains 1 mM dNTPs, 3 mM $MgCl_2$ (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 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 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 (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 50 pg-10 ng DNA per 50 μL reaction volume. For eukaryotic genomic DNA, we recommend a starting amount of 200 ng DNA per 50μL 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^{2+}.

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 suitable for GC-rich templates. Increasing this step up 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 Tm 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 sufficient for amplicons under 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.

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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 3 min
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 45 s/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@meridianlifescience.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

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