SimpliFi HS Mix

Shipping: On Dry/Blue Ice

Catalog numbers: BIO-25060 : 100 x 50 µL reactions (2 x 1.25 mL)
BIO-25061 : 500 x 50 µL reactions (10 x 1.25 mL)

Batch No.: See vial

Concentration: 2x

Store at –20 °C

meridian BIOSCIENCE™

Storage and stability:
SimpliFi HS Mix is shipped on dry/blue ice. On arrival store at -20 °C for optimum stability. Repeated freeze/thaw cycles should be avoided. Thawing during transportation does not affect the product performance. Solutions should be mixed/equilibrated after each thawing to avoid phasing.

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:
Read and understand the SDS (Safety Data Sheets) before handling the reagents. Hardcopies of the SDSs will be provided with the first shipment, thereafter they will be available upon request.

Quality control:
Meridian operates under ISO 13485 Management System. SimpliFi HS Mix and its components are extensively tested for activity, processivity, efficiency, heat activation, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination.

Notes:
For research and further manufacturing use only.

Features

- High fidelity coupled with high yield
- Inhibitor-tolerant, amplifying of a broad range of targets
- Low GC bias
- Convenient pre-mixed, pre-optimized 2x solution
- Reproducible results

Applications

- Ideal for crude samples such as blood
- NGS library amplification
- Multiplex PCR
- Blunt-end cloning

Description

SimpliFi HS Mix is a convenient ready-to-go 2x reaction mix combining the latest advances in buffer chemistry and PCR enhancers and stabilizers, together with an aptamer-mediated hot-start polymerase, dNTPs and $MgCl_2$. It has been designed for highly reproducible, accurate assay results in the presence of inhibitors. The mix is optimized and ready-to-use, the user is simply required to add water, template and primers.

The advanced buffer chemistry and enhancers in SimpliFi HS Mix have been developed for fast PCR and is designed for superior sensitivity and specificity. SimpliFi HS Mix has been developed to reduce GC bias, making it perfect for NGS library amplification.

Components

Component	100 reactions	500 reactions
SimpliFi™ HS Mix	2 x 1.25 mL	10 x 1.25 mL

Standard SimpliFi HS 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:

SimpliFi™ HS Mix, 2x	25 µL
Primers 20 µM each	1 µL
Template	as required
Water (ddH_2O)	up to 50 µL

PCR cycling conditions:

Step	Temperature	Time	Cycles
Initial denaturation	95 °C	30 s	**1**
Denaturation	95 °C	15 s	25-35
Annealing	User determined*	15 s	
Extension	72 °C	15 - 30 sec/kb	
Final extension (optional)	72 °C	4 - 10 min	1

*Annealing temperature is primer dependent

Important considerations and PCR optimization

The optimal conditions will vary from reaction to reaction and are dependent on the template/primers used.

Mg^{2+} concentration: The Mg^{2+} concentration in the 2x mix is 4 mM (2 mM final concentration), this is the optimum concentration for SimpliFi HS Mix for most PCR reactions and should only be adjusted if necessary. Additional Mg (up to 4 mM in the final reaction) should be added in presence of more than 10% of whole blood.

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 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). 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 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^{2+}.

Multiplexing: For multiplex PCR we suggest using 55 °C as a starting annealing temperature. If further optimization is required we recommend using a temperature gradient to determine the optimal annealing temperature needed for the multiplex PCR. Since multiplex PCR generally requires a longer extension step, we suggest starting with a minimum of 90 s and increasing it if required.

Website: www.bioline.com/ email: info@meridianlifescience.com

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 e.g. GC or AT-rich, or high level of secondary structure	- Increase initial denaturation time to 5 minutes - Increase 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
	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

Technical Support

For any technical enquiries, please contact our Technical Support team via email at: mbi.tech@meridianlifescience.com

Associated Products

Product Name	Pack Size	Cat. No.
ACCUZYME™ Mix	500 reactions	BIO-25028
JetSeq™ ER and Ligation Kit	96 reactions	BIO-68026

Bioline Reagents Ltd
UNITED KINGDOM
Tel: +44 (0)20 8830 5300
Fax: +44 (0)20 8452 2822

Meridian Life Science Inc.
USA
Tel: +1 901 382 8716
Fax: +1 901 382 0027

Bioline GmbH
GERMANY
Tel: +49 (0)337 168 1229
Fax: +49 (0)3371 68 1244

Bioline (Aust) Pty. Ltd
AUSTRALIA
Tel: +61 (0)2 9209 4180
Fax: +61 (0)2 9209 4763

Website: www.bioline.com/ email: info@meridianlifescience.com