LifeSct.com
Life Scientists' Service Center
Email: support@lifesct.com
Tel: 240-715-2985 Fax: 240-252-7376
14323 Woodcrest Dr., Rockville, MD 20853

2× LiSpark™ Max SuFi PCR Master Mix

Cat. #: M0021 Size: 1 ml/5 ml/15 ml

Introduction

2× LiSpark™ Max SuFi PCR Master Mix is a mix of LiSpark™ Max Super-Fidelity DNA Polymerase at 2x concentration, boasting a fidelity 53x that of the LiTaq™ DNA polymerase. It features 2 monoclonal antibodies for hot start PCR, with our proprietary extension enhancer, specificity enhancer and inhibition relief factor added, resulting in the most robust polymerase on market. The protective agent in the system guarantees that LiSpark™ Max SuFi PCR Master Mix can keep its activity even after many freezing and thawing cycles. This product is capable of amplifying 20kb genomic DNA and 40kb λDNA, and will generate blunt ends compatible with ***LiClone™ One Step DNA Assembly Kit*** (Cat. #: M0031).

Package Information

Components	M0021-01	M0021-05	M0021-15
2× LiSpark™ Max SuFi PCR Master Mix	1 ml	5x1 ml	15x1 ml

Storage

All materials should be stored at -20°C.

Protocol

All components should be mixed as recommended below prior to use.

Components	25µl Reaction Volume	50µl Reaction Volume
2× LiSpark™ Max SuFi PCR Master Mix	12.5 µl	25 µl
DNA template (100 ng / µl)	variable	variable
Upstream primer (10 µM)	1 µl	2 µl
Downstream primer (10 µM)	1 µl	2 µl
Distilled water (dH_2O)	To 25 µl	To 50 µl
Total reaction volume	25 µl	50 µl

* The recommendation for final enzyme concentration is1 U/ 50 µl, but it can be varied in a range of 0.5 – 2 U/ 50 µl, if needed

Note:

a. Add the components into sterile PCR tubes while mixing gently.

b. Place PCR tubes to a PCR cycler.

c. Perform PCR reaction using optimized cycling conditions. Suggested cycling parameters for using LiSpark™ Max Super-Fidelity DNA Polymerase are provided below. Analyze PCR amplification products on a 0.7–1.0% (w/ v) agarose gel.

3-step protocol

Segment	Number of cycles	Temperature	Duration
Initial Denaturation	1	95°C	3 min (30 sec)
PCR	25-35	95°C	15 sec
		55-65°C	15 sec
		72°C	15-30 sec/kb
Final Extension	1	72°C	5 min
Hold	1	4°C	∞

2-step protocol

Segment	Number of cycles	Temperature	Duration
Initial Denaturation	1	95°C	3 min (30 sec)
PCR	25-35	95°C	15 sec
		72°C	15-30 sec/kb
Final Extension	1	72°C	5 min
Hold	1	4°C	∞

Notes:

a. This mix is based on a hot-start DNA polymerase, the pre-denaturation activation condition should be set to 95 °C for 3 minutes (for genomic DNA and cDNA) or 30sec (for plasmid DNA and virus DNA) to thoroughly activate the enzyme.

b. Optimized cycling parameters may not necessarily be transferable between thermal cyclers. Consult the instrument manufacturer's recommendations if further optimization of cycling parameters is required.

c. The annealing temperature set up should be based on the Tm of the primers.

d. For primers with annealing temperatures ≥ 72°C, a 2-step protocol is recommended.

Trouble Shooting

No product at all or low yield

1. High quality or purified DNA templates are preferred to enhance the success of PCR.

LifeSct.com
Life Scientists' Service Center
Email: support@lifesct.com
Tel: 240-715-2985 Fax: 240-252-7376
14323 Woodcrest Dr., Rockville, MD 20853

2× LiSpark™ Max SuFi PCR Master Mix

Cat. #: M0021 Size: 1 ml/5 ml/15 ml

2. Repeat and make sure that there are no pipetting errors.
3. Use fresh high quality dNTPs.
4. Do not use dNTP mix or primers that contain dUTP or dITP.
5. Sample concentration may be too low. Use more templates.
6. Template DNA may be damaged. Use carefully purified template and make sure template is not fragmented.
7. Increase extension time.
8. Increase the number of cycles.
9. Optimize annealing temperature
10. Optimize enzyme concentration.
11. Optimize the denaturation time.
12. Check the purity and concentration of the primers.
13. Check primer design.

Non-specific products - High molecular weight smears

1. Decrease enzyme concentration.
2. Decrease extension time.
3. Reduce the total number of cycles.
4. increase annealing temperature or try 2-step protocol.
5. Vary denaturation temperature.
6. Decrease primer concentration.

Non-specific products - High molecular weight smears

1. Increase annealing temperature.
2. Decrease extension time.
3. Decrease enzyme concentration.
4. Titrate template amount.
5. Decrease primer concentration

Limited Product Warranty

This warranty limits our liability to replacement of this product. No other warranties of any kind, express or implied, including without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by LifeSct.com. We shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

MSDS

Material safety data sheet (MSDS) is available at www.lifesct.com.

FOR RESEARCH USE ONLY