LifeSct.com
Life Scientists' Service Center

Email: support@lifesct.com
Tel: 240-715-2985 Fax: 240-252-7376
9610 Medical Center Drive, Rockville, MD 20850

LiSpark™ Ultra SuFi DNA Polymerase

Cat. #: M0030 Size: 100/500 U

Introduction

LiSpark™ Ultra SuFi DNA Polymerase is a fast, high-efficiency, high-fidelity DNA polymerase with 5'-3' DNA polymerase activity and 3'-5' exonuclease activity. This polymerase is modified from other high-fidelity enzymes, has strong amplification ability, rapid amplification speed (4-6 kb/min), and high fidelity. This polymerase overcomes some defects of Pfu polymerase such as the poor amplification ability, low yield and amplification rate, which greatly shortens the reaction time.

This product can be used for the amplification of long fragments and other various complex templates. The PCR product does not have an "A" base at the 3' end and can be directly used for blunt-end cloning. For T/A cloning, it is necessary to add "A" to the end of the PCR product. This product has the characteristics of rapid extension, high amplification efficiency and high fidelity. It is suitable for experiments such as gene cloning, site-directed mutagenesis, and SNP amplification.

Package Information

Components	M0030-01 (100 U)	M0030-01 (500 U)
LiSpark™ Ultra SuFi DNA Polymerase (2 U/μl)	50 μl	5×50 μl
5× LiSpark™ HF Buffer	1.5 ml	5×1.5 ml
5× LiSpark™ GC Buffer	1.5 ml	5×1.5 ml
dNTP Mix, 10 mM each	100 μl	5×100 μl

The amount of enzyme required to incorporate 10 nmol deoxynucleotide into the acidic insoluble material within 30 minutes at 74°C is defined as 1 activity unit (U).

Storage

All materials should be stored at -20°C.

Recommended PCR Reaction System

5× LiSpark™ HF Buffer	10 μl	1x
dNTP Mix, 10 mM each	1 μl	200 μM each
Template DNA	Optional	< 250 ng/50 μl
Forward Primer, 10 uM	2.5 μl	0.5 μM
Reverse Primer, 10 uM)	2.5 μl	0.5 μM
LiSpark™ Ultra SuFi DNA Polymerase	0.5 μl	1 U/50 μl
ddH_2O	to 50 μl	

Note:

1. The 5× LifeSct™ HF Buffer and 5× LifeSct™ GC Buffer contain 7.5 mM Mg^{2+}.

2. It is recommended to use 5× LifeSct™ GC Buffer for complex templates and templates with high GC content.

Recommended PCR Reaction Program

Steps	Temperature	Time	Cycles
Pre-denaturation	98°C	30 sec-3 min	1
Denaturation	98°C	5-10 sec	25-35
Annealing	45-72°C	10-30 sec	
Extension	72°C	2-4 kb/min	
Final Extension	72°C	5-10 mins	1

Note:

1) Denaturation: For simple DNA templates, the pre-denaturation temperature is 98°C and the pre-denaturation time is 30 s to 1 minute. For more complicated templates, the pre-denaturation time can be extended to 3 minutes.

2) Annealing: the annealing temperature should be the 3-5°C lower than the Tm of primer. If the ideal amplification efficiency cannot be obtained, the annealing temperature should be changed in a gradient to optimize. When non-specific reactions occur, the annealing temperature should be appropriately 25-35 cycles increased. Two-step PCR can be used for primers with high Tm.

3) Extension: The extension time should be set according to the length of the amplified fragment and the complexity of the template. The amplification efficiency of the LifeSct™ DNA Polymerase is 4-6 kb/min. For simple templates, the rate can be 6 kb/min.

4) Cycles: The number of cycles can be set based on the downstream applications of the PCR product. If the number is too low, the amount of PCR product is insufficient; if the number is high, the probability of mismatch and the non-specific background are increased. Therefore, the number of cycles should be reduced as much as possible yet ensuring the yield of the product.

Primers Designing Notes

1. Choose C or G as the last base of the 3'-end of the primer.

2. Avoid continuous mismatching at the last 8 bases of the 3'-end of the primer.

3. Avoid hairpin structure at the 3'-end of the primer.

4. T_m of the primers should be within the range of 55°C-65°C.

5. Additional sequence should not be included when calculating T_m of the primers.

6. GC content of the primers should be within the range of 40% - 60%.

7. T_m and GC content of forward and reverse primes should be as similar as possible.

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