FuniCut™ SpeI

Product description

The FuniCut™ series of restriction endonucleases are rapid enzymes produced through genetic engineering recombinant technology, capable of precisely completing DNA cleavage within 5 to 15 minutes. They are suitable for the rapid digestion of plasmid DNA, PCR products, genomic DNA, and more. The FuniCut™ series of rapid restriction enzymes share a common digestion buffer, simplifying the reaction system. Additionally, they exhibit excellent enzyme activity redundancy, easily handling substrate excess or challenging templates.

Specifications

Cat NO.	15028ES50
Size	50 T

Components

Components No.	Name	15028ES50
15028-A	FuniCut™ SpeI	50 µL
15028-B	10xFuniCut™ Buffer	1 mL
15028-C	10xFuniCut™ Color Buffer	1 mL

Storage

Shipped with ice packs.This product should be stored at -25°C ~ -15°Cfor 2 years.

Recognition Site

5'-A↓CTAGT-3'

3'-TGATC↑A-5'

Recommended Reaction Conditions

1x FuniCut™ Buffer;

optimal incubation at 37°C.

Inactivation Conditions

Incubate at 80°C for 20 minutes.

Notes

1. No star activity observed after a 3-hour incubation; extended digestion may lead to star activity.
2. Sequences may overlap due to EcoKI methylation, affecting cleavage.
3. Sequences may overlap due to EcoBI methylation, affecting cleavage.
4. For your safety and health, wear lab coat and disposable gloves during operation.
5. This product is for research use only!

Quality Control

1. Functional Activity Test: Under optimal reaction conditions in a 20 µL reaction system, 1 µL of enzyme can completely digest 1 µg pUC19-SpeI DNA within 15 minutes.

2. Extended Incubation Test: Under optimal reaction conditions, incubating 1 µL of enzyme with 1 µg pUC19-SpeI DNA for 3 hours did not detect any non-specific degradation caused by contaminating nucleases or star activity. However, prolonged incubation may result in star activity.

3. Digestion-Ligation-Restriction Test: Under optimal reaction conditions, using 1 µL of enzyme to digest the substrate, recovering the digested product, and ligating it with an appropriate amount of T4 DNA ligase at 22°C, the ligation product can be redigested with the same enzyme.

4. Non-specific Endonuclease Activity Test: Under optimal reaction conditions, incubating 1 µL of enzyme with 1µg supercoiled plasmid DNA for 4 hours showed that the plasmid DNA remained in its supercoiled state upon agarose gel electrophoresis.

5. Blue-White Screening: Transform competent E. coli cells with re-ligated products from vectors containing a single lacZa gene digested with 1 µL of enzyme. Correctly ligated products will grow as blue colonies on LB plates containing the corresponding antibiotic, IPTG, and X-gal. Incorrectly ligated products (i.e., incomplete digestion) will produce white colonies. The proportion of white colonies should be less than 1% for the FuniCut™ series of restriction enzymes.

Instructions

1. Rapid DNA Digestion Procedure

1) Prepare the reaction mixture following the suggested order of addition (operate on ice):

Component	Plasmid DNA	PCR Product	Genomic DNA
ddH_2O	15 µL	16 µL	30 µL
10xFuniCut™ Buffer or 10xFuniCut™ Color Buffer	2 µL	3 µL*	5 µL
Substrate DNA	2 µL (~1 µg)	10 µL (~0.2 µg)	10 µL (5 µg)
FuniCut™ SpeI	1 µL	1 µL	5 µL
Total	20 µL	30 µL	50 µL

[Note]:*This system refers to purified PCR products. Unpurified PCR products possess certain ionic strength, thus the volume of 10xFuniCut™ Buffer can be reduced to 2µL accordingly. If proceeding with cloning experiments, purify PCR products before digestion.

2) Gently mix by pipetting or flicking the tube wall (do not vortex), then briefly centrifuge to collect droplets from the tube walls.

3) Incubate at 37°C for 15 minutes (plasmids), or 15 to 30 minutes (PCR products), or 30 to 60 minutes (genomic DNA).

4) Incubate at 80°C for 20 minutes to deactivate the enzyme and stop the reaction (optional).

2. Double or Multiple Digests

1) Use 1 µL of each endonuclease, appropriately increasing the reaction volume as needed.

2) The total volume of all enzymes should not exceed one-tenth of the total reaction volume.

3) If the optimal reaction temperatures for the selected enzymes differ, start digestion with the lower temperature enzyme first, then add the higher temperature enzyme, incubating at the higher temperature thereafter.

3.Scaling Up Plasmid Reactions

Component	Volume (20 µL)	Volume (20 µL)	Volume (50 µL)
DNA	1 µg	2 µg	5 µg
FuniCut™ SpeI	1 µL	2 µL	5 µL
10xFuniCut™ Buffer or 10xFuniCut™ Color Buffer	2 µL	2 µL	5 µL
Total	20 µL	20 µL	50 µL

[Note]: For total reaction volumes greater than 20 µL, water baths, metal baths, or sand baths can be used, along with increased incubation times.

Recognition Sites in Different DNAs

λDNA	ΦX174	pBR322	pUC57	pUC18/19	SV40	M13mp18/19	Adeno2
0	0	0	0	0	0	0	3

Methylation Effects

Dam	Dcm	CpG	EcoKI	EcoBI
No effect	No effect	No effect	Sequences may overlap, affecting cleavage	Sequences may overlap, affecting cleavage

Activity in Different Reaction Buffers

Reaction Buffer	FuniCut™ Buffer	Thermo Scientific FastDigest Buffer	NEB CutSmart™ Buffer	Takara QuickCut™ Buffer
Activity	100%	100%	100%	100%

[Note]: Activity data derived from standard reaction system tests conducted by Yeasen Biological.

Isoschizomers

AhlI, BcuI

[Note]: Isoschizomers may have different sensitivities to various methylations.