FuniCut™ Esp3I (BsmBI)

Product description

The FuniCut™ series of restriction endonucleases are rapidly acting, genetically engineered enzymes capable of precisely cutting DNA within 5 to 15 minutes. They are ideal for the rapid digestion of plasmid DNA, PCR products, genomic DNA, and more. The FuniCut™ series shares a universal digestion buffer, simplifying the reaction setup, and offers excellent enzyme activity redundancy, making it easy to handle substrate excess or challenging templates.

Specifications

Cat NO.	15048ES30
Size	30 T

Components

Components No.	Name	15048ES30
15048-A	FuniCut™ Esp3I (BsmBI)	30 µL
15048-B	10×FuniCut™ Buffer	1 mL
15048-C	10×FuniCut™ Color Buffer	1 mL

Storage

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

Recognition Site

5'-CGTCTC(N)1↓-3'

3'-GCAGAG(N)5↑-5'

Recommended Reaction Conditions

1× FuniCut™ buffer; incubate at 37°C.

Inactivation Conditions

Incubate at 80°C for 20 minutes.

Notes

1. No star activity observed after a 3-hour incubation; however, prolonged digestion may lead to star activity.
2. Affected by CpG methylation; if the sequence overlaps completely, cleavage is blocked.
3. Affected by EcoBI methylation; if the sequence overlaps, cleavage may be affected.
4. For safety, please wear lab coat and disposable gloves during operation.
5. This product is for research use only!

Quality Control

1. Functional Activity: Under optimal conditions, 1 µL of enzyme can fully digest 1 µg λDNA in 15 minutes in a 20 µL reaction volume.

2. Extended Incubation Test: After co-incubating 1 µL enzyme with 1 µg λDNA for 3 hours under optimal conditions, no non-specific degradation due to contamination or star activity was detected.

3. Digestion-Ligation-Restriction Test: Digestion, ligation, and re-digestion were successfully performed without any issues.

4. DNase Residual Detection: After incubating 1 µL FuniCut™ Esp3I with double-stranded DNA substrate at 37°C for 16 hours, no changes were observed in the double-stranded DNA substrate through gel electrophoresis.

Instructions

1. Rapid DNA Digestion Protocol

1) Prepare the reaction mixture as follows (operate on ice):

Component	Plasmid DNA	PCR Products	Genomic DNA
ddH_2O	15 µL	16 µL	30 µL
10×FuniCut™ Buffer or 10×FuniCut™ Color Buffer	2 µL	3 µL*	5 µL
Substrate DNA	2 µL(~1 µg)	10 µL (~0.2 µg)	10 µL (5 µg)
FuniCut™ Esp3I (BsmBI)	1 µL	1 µL	5 µL
Total	20 µL	30 µL	50 µL

[Note]: *This system refers to purified PCR products. Unpurified PCR products contain a certain ionic strength; therefore, the amount of 10×FuniCut™ Buffer can be appropriately reduced to 2 µL.

If the digested product will be used for downstream applications such as cloning, the PCR product should be purified before digestion.

2) Gently mix by pipetting or flicking the tube wall (avoid vortexing), then briefly centrifuge to collect droplets.

3) Incubate at 37°C for 15 min (plasmid), 15-30 min (PCR products), or 30-60 min (genomic DNA).

4) Optionally, inactivate the enzyme by incubating at 80°C for 20 minutes.

2. Double or Multiple Digestions

1) Use 1 µL of each enzyme, adjusting the total reaction volume accordingly.

2) The total volume of all enzymes should not exceed 1/10th of the total reaction volume.

3) If using enzymes with different optimal temperatures, start with the lower temperature enzyme before adding the higher temperature one.

3. Scaling Up Plasmid Reactions

Component	Volume (20 µL)	Volume (20 µL)	Volume(50 µL)
DNA	1 µg	2 µg	5 µg
FuniCut™ Esp3I	1 µL	2 µL	5 µL
10×FuniCut™ Buffer or 10×FuniCut™ Color Buffer	2 µL	2 µL	5 µL
Total	20 µL	20 µL	50 µL

[Note]: For reactions larger than 20 µL, consider water bath, metal bath, or sand bath, with extended incubation time.

Number of Recognition Sites in Different DNAs

λDNA	ΦX174	pBR322	pUC57	pUC18/19	SV40	M13mp18/19	Adeno2
14	0	1	2	2	0	1	21

Methylation Sensitivity

Dam	Dcm	CpG	EcoKI	EcoBI
No effect	No effect	Complete overlap leads to blockage	No effect	Sequence overlap may affect cleavage

Enzyme Activity in Different Buffers

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

[Note]: Activity data obtained from standard reaction conditions according to Yeasen Bio's testing protocols.

Isoschizomers

BsmBI, BstGZ53I, Esp16I, Esp23I

[Note]: Sensitivity to methylation may vary among these isoschizomers.