Esp3I(BsmBI), ADCF

REF: EG21504-S/H

Note: ADCF (animal derived component free)

Isoschizomers*: BsmBI

Note: *Isoschizomers may have different methylation sensitivitives.

Storage Condition

-20℃

Components

Component	EG21504S	EG21504H
Esp3I(BsmBI), ADCF	2000 U (10 U/μl)	2000 U (50 U/μl)
10× Esp3I Buffer	4×1.25 ml	4×1.25 ml

Description

Esp3I(BsmBI), ADCF, is a genetically engineered recombinant enzyme that can accurately cleave plasmid DNA, PCR products, or genomic DNA in 15 min~1 h.

This product is manufactured without the use of animal-derived components or antibiotics throughout the fermentation, purification, and formulation processes.

Definition of Activity Unit

One unit is defined as the amount of enzyme required to completely cleave 1 μg of λDNA in a 50 μl reaction system at 37℃ for 1 hour.

Quality Control Assays

Protein Purity

The enzyme is ≥95% pure as determined by SDS-PAGE analysis using Coomassie Blue staining.

DNase Activity

A 20 μl reaction containing 15 ng of dsDNA fragments and 50 U of Esp3I(BsmBI), ADCF incubated for 16 hours at 37 ℃ results in no detectable degradation of the dsDNA fragments as determined by agarose gel electrophoresis.

RNase Activity

A 10 μl reaction containing 500 ng of total RNA and 50 U of Esp3I(BsmBI), ADCF incubated for 1 hours at 37 ℃ results in >90% of the substrate RNA remains intact as determined by agarose gel electrophoresis.

Ligation and Recuting

Under optimal reaction temperature, digest the substrate using 50 U Esp3I(BsmBI), ADCF and recover the digested products. >95% of the DNA fragments can be ligated with T4 DNA Ligase at 22℃ .Of these ligated fragments, >95% can be recut with BsmBI as determined by agarose gel electrophoresis.

Host Cell DNA

Residual nucleic acids in the enzyme solution were detected using TaqMan qPCR specific to *E.coli* 16S rDNA. The residual *E.coli* genomic DNA was found to be less than 10 pg.

Host Cell Protein

The content of host proteins derived from *E. coli* is ≤50 ppm as determined by ELISA.

Microbial Limit

This product is tested by the microbial count method, and the total aerobic microbial count is below 5 cfu/ml, and the total combined yeasts/molds count is below 5 cfu/ml.

Bacterial Endotoxin

The residual bacterial endotoxin in the product is <1 EU/KU.

Icon Descriptions

 The enzyme's optimum reaction temperature is 37℃ .

 Cleavage with this restriction enzyme may be blocked or impaired when the substrate DNA is methylated by the CpG methylase.

 Inactivation condition is incubation at 80℃ for 20 minutes.

 3 hours incubation do not show star activity, but longer incubation may result in star activity.

 Animal derived component free.

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Protocol

Protocol for DNA Digestion

① Combine the following components on ice in the following order:

Reagents	Volume
ddH_2O	up to 50 μl
10× Esp3I Buffer	5 μl
DNA[a]	1 μg
Esp3I(BsmBI), ADCF	5~10 U
Total	50 μl

a. DNA substrates should contain no phenol, chloroform, ethanol, EDTA, detergents, or high salt concentrations, otherwise enzyme activity will be affected; methylated DNA can inhibit the cutting reaction of certain restriction endonucleases.

② Mix gently and spin down;
③ Incubation at 37℃ for 15 minutes~1 hour, generally recommended 5 U~10 U enzyme/μg DNA, 10 U~20 U enzyme/μg genomic DNA, warm bath for 1 hour, if you need to overnight digestion reaction, please adjust the enzyme amount to 2.5 U;
④ Optional: Inactivate the enzyme by heating at 80℃ for 20 minutes, or by adsorption column or phenol/chloroform purification to terminate the reaction.
⑤ The volume of enzyme added to the reaction mixture should not exceed 10% of the total volume to avoid star activity caused by excessive glycerol in the enzyme storage buffer.
⑥ The additives (e.g., glycerol, salt) in the enzyme storage buffer are the same as the contaminants in the substrate solution (e.g., salt, EDTA, or ethanol, etc.). Therefore, the smaller the reaction volume, the stronger the digestion inhibition effect.
⑦ Recommended reaction systems for small-scale reactions:

DNA	0.1 μg	0.5 μg
Esp3I(BsmBI), ADCF	1 U	5 U
10× Esp3I Buffer	1 μl	2.5 μl
Total	10 μl[b]	25 μl

b. To prevent evaporation, the incubation time for a 10 μl reaction system should not exceed 1 hour.

Number of Recognition Sites in DNA

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

Methylation Effects on Digestion

Dam	Dcm	CpG	EcoKI	EcoBI
No effect	No effect	Impaired	No effect	No effect

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