EcoRI, ADCF

REF: EG22504S

Note: ADCF (animal derived component free)

Isoschizomers*: FunII

Note: The sensitivity of FunII restriction enzyme may vary depending on different methylation modifications.

Storage Condition

-20℃

Components

Component	Amount
EcoRI, ADCF (20 U/μl)	5000 U
10× CutOne® Buffer (ADCF)	2×1 ml

Description

EcoRI, 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 digest 1 μg of λDNA in a 50 μl reaction system at 37℃ for 1 hour.

Quality Control Assays

Protein Purity

The purity of this product is ≥95% as determined by SDS-PAGE.

Non-specific Endonuclease Activity

A 50 μl reaction containing 1 μg of supercoiled plasmid and 20 U of EcoRI, ADCF incubated for 4 hours at 37℃ results in <10% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

Blue/White Screening

An appropriate vector containing *lacZα* gene is digested by 1 μl EcoRI, ADCF. The digested product is ligated and transformed into *E.coli* competent cell. On Luria-Bertani culture plate with X-Gal, IPTG and appropriate antibiotic, the successfully ligated β-galactosidase gene can be expressed and gives rise to a blue colony, while an interrupted gene (i.e. degraded DNA end) gives rise to a white colony. ADCF restriction enzymes must produce fewer than 1% white colonies.

DNase Activity

A 20 μl reaction containing 15 ng of dsDNA fragments and 20 U of EcoRI, 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 RNA and 20 U of EcoRI, 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 20 U EcoRI, 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 *E.coli* host proteins in the product was determined to be ≤ 50 ppm using the ELISA method.

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 < 0.5 EU/KU.

Icon Descriptions

37 The enzyme's optimum reaction temperature is 37℃ .

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

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

Add: No.17 Huaguoshan Avenue,Lianyungang City, China
Tel: +86 (518) 8558 6628 • E-mail:support@best-enzymes.com • www.best-enzymes.com

Protocol

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

Reagents	Volume
ddH_2O	Up To 50 μl
10× CutOne® Buffer (ADCF)	5 μl
DNA[a]	1 μg
EcoRI, ADCF	10~20 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 1 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
EcoRI, ADCF	1 U	5 U
10× CutOne® Buffer (ADCF)	1 μl	2.5 μl
Total	10 μl[b]	25 μl

b. To prevent evaporation, 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
5	0	1	1	1	1	1	5

Methylation Effects on Digestion

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

Add: No.17 Huaguoshan Avenue,Lianyungang City, China
Tel: +86 (518) 8558 6628 • E-mail:support@best-enzymes.com • www.best-enzymes.com