XhoI, GMP Grade

REF: GMP504S

Isoschizomers*: PaeR7I, Sfr274I, SlaI

*Isoschizomers may have different methylation sensitivities.

Storage and Transportation Condition

Store at -20 ± 5℃ , Valid for 24 months. Transport at ≤ 0℃ .

Components

Component	Amount
XhoI, GMP Grade (20 U/μl)	1 ml
10× Cut Buffer F, GMP Grade	6×1 ml

Description

XhoI, GMP Grade, is obtained by recombinant expression in *Escherichia coli* and can precisely digest target DNA within 15 minutes to 1 hour. This product is manufactured and quality-controlled in compliance with GMP specifications, ensuring full traceability of the production process and raw materials. The entire production process does not involve the use of antibiotics or any animal-derived materials and excipients. Stringent controls are implemented for process-related impurities including host proteins, exogenous DNA, non-specific endonucleases, DNase, RNase, as well as microbial limits and bacterial endotoxins. This product meets the requirements for raw materials in fields such as vaccine and pharmaceutical production.

Definition of Activity Unit

One unit is defined as the amount of enzyme required to completely digest 1 μg of λDNA (HindIII digest) in 1 hour at 37℃ in a reaction volume of 50 μl.

Quality Control Assays

Protein Purity

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

Star Activity

A 50 μl reaction in Cut Buffer F, GMP Grade containing 1 μg of λDNA (HindIII digest) and 20 U of this product incubated for 1 hours at 37 ℃ results in a DNA pattern free of detectable degradation by other nuclease or star activity as determined by agarose gel electrophoresis. Longer incubation may result in star activity.

Non-specific Endonuclease Activity

A 50 μl reaction in Cut Buffer F, GMP Grade containing 1 μg of supercoiled plasmid and 20 U of this product incubated for 4 hours at 37 ℃ results in <20% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

DNase Activity

A 20 μl reaction in Cut Buffer F, GMP Grade containing 15 ng of dsDNA fragments and 20 U of this product 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 in Cut Buffer F, GMP Grade containing 500 ng of RNA and 20 U of this product incubated for 1 hours at 37 ℃ results in >90% of the substrate RNA remains intact as determined by agarose gel electrophoresis.

Host Cell DNA

Using the third method of qPCR specified in General Chapter 3407 of ChP(2020) Volume IV, the residual *Escherichia coli* host cell DNA content of this product is below 10 copies/20 U.

Host Cell Protein

Using the method specified in General Chapter 3412 of ChP(2020) Volume IV, the residual *Escherichia coli* host cell protein content of this product is below 50 ppm.

Microbial Limit

Using the method specified in General Chapter 1105 of ChP(2020) Volume IV, the total aerobic microbial count of this product is below 5 cfu/ml, and the total combined yeasts/molds count is below 5 cfu/ml.

Bacterial Endotoxin

Using the first method of gel-clot specified in General Chapter 1143 of ChP(2020) Volume IV, the residual bacterial endotoxin content of this product is below 0.5 EU/KU.

Mycoplasma

Using a Mycoplasma detection kit (LAMP method) to test 20 U of this product, the result was negative.

Heavy Metals

Using the first method specified in General Chapter 0821 of ChP(2020) Volume IV, the residual heavy metals content of this product is below 10 ppm.

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.

80 The enzyme can be inactivated by incubating at 80℃ for 20 minutes.

★ Star activity will not occur following the recommended reaction conditions. Extended incubations and/or high concentrations of this enzyme may result in star activity.

Animal derived component free.

GMP Manufactured in compliance with GMP specifications.

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Protocol

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

Reagents	Volume
ddH_2O	up to 50 µl
10× Cut Buffer F, GMP Grade	5 µl
DNA[a]	1 µg
XhoI, GMP Grade (20 U/µl)	1 µl
Total	50 µl

a. DNA substrates should contain no phenol, chloroform, ethanol, EDTA, detergents, or high salt concentrations, otherwise enzyme activity will be affected;

② Mix gently and spin down.
③ Incubate at 37℃ for 15 minutes~1 hour.
④ Inactivate the enzyme by heating at 80℃ for 20 minutes.

Notice

① 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.
② 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.

Number of Recognition Sites in Different DNA

λDNA	ΦX174	pBR322	pUC57	pUC18/19	SV40	M13mp18/19	Adeno2
1	1	0	0	0	0	0	6

Methylation Effects on Digestion

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

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