XbaI, Animal Free

Cat #: C-BSM2502S

Size: 5000 U

Storage: -20°C

5'...T▼C T A G A...3'
3'...A G A T C▲T...5'

Note:ADCF(animal derived component free), animal-derived component free Free

Components

Components	Amount
XbaI, ADCF	5000 U
10× XbaI Buffer	2×1 ml

Description

XbaI, ADCF, which has been genetically engineered, is able to digest DNA in 5~15 minutes. It can be used to digest plasmid DNA, PCR products, and genomic. XbaI, ADCF has the following characteristics: rapid digestion within 5 to 15 minutes, excellent enzyme activity redundancy, easy handling of substrate excess or challenging template digestion. This enzyme is animal-derived component free.

Unit Definition

One unit is defined as the amount of XbaI required to digest 1 µg of λDNA (Dam$^-$ /HindIII digest) in 1 hour at 37°C in 50 µL of recommended reaction buffer.

Heat Inactivation

Incubation at 80°C for 20 minutes.

Quality Control

Purification

Greater than 95% by SDS-PAGE.

Endonuclease residues

Incubate 20 U of XbaI with supercoiled plasmid DNA at 37°C for 4 hours, no changes for the plasmid were detected in DNA electrophoresis.

Blue/White Screening Assay

An appropriate vector containing *lacZα* gene is digested by 1 μl XbaI. 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 residues

Incubate 20 U of XbaI with double-stranded DNA at 37°C for 16 hours, no changes for the DNA were detected in DNA electrophoresis.

RNase residues

Incubate 20 U of XbaI with RNA at 37°C for 1 hour, no degradation for the RNA was detected in electrophoresis.

Ligation and Recuting

After digestion with 20 U of XbaI at optimal reaction temperature, the DNA fragments can be ligated with T4 DNA Ligase (Fast) at 22°C. Of these ligated fragments, most of them can be recut with BsaI as determined by agarose gel electrophoresis.

Host DNA residues

The nucleic acid residues in the enzyme solution were detected by TaqMan qPCR specific for *E.coli* 16S rDNA, less than 10 pg *E.coli* genome residues were detected.

Host Protein residues

The *E.coli* host protein was determined by ELISA to be less than 50 ppm.

Sterility

No *E. coli* was detected.

Endotoxin residues

< 10 EU/mg

Icon Descriptions

This enzyme will digest unit substrate in 5~15 minutes under recommended reaction conditions.

The enzyme's optimum reaction temperature is 37°C.

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

The enzyme can be heat inactivated at by incubation 80°C for 20 minutes.

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

This enzyme is animal-derived component free.

Method of application

Protocol for Fast DNA Digestion

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

	DNA
ddH_2O	Up To 50 µl
10× XbaI Buffer	5 µl
DNA[a]	1 µg
XbaI, ADCF	10-20 U
Total	50 µl

a. The DNA substrate should not contain phenol, chloroform, ethanol, EDTA, detergent or high concentration salt, otherwise it will affect the enzyme activities of XbaI, ADCF; Methylated DNA will inhibit digestion for certain restriction enzymes.

② Mix gently(Do not vortex) and spin down;

③ Incubate at 37°C for 15 min-60 min, generally recommended 5~10 U enzyme /μg DNA, 10~20 U enzyme /μg genomic DNA, warm bath for 1 h, if overnight enzyme digestion is required, please adjust the enzyme amount to 1U;

④ Inactivate the enzyme by heating for 20 minutes at 80°C, Or terminate the reaction by column-based or phenol/chloroform purification.

⑤ The volume of enzyme added to the reaction should not exceed 10% of the total volume to avoid excessive glycerol, which may lead to star activity.

⑥ The additives in the storage buffer of restriction endonucleases (such as glycerol and salts) and contaminants in the substrate (such as salts, EDTA, or ethanol, etc.) are similar, the smaller the reaction volume, the stronger the inhibitory on the enzyme digestion reaction.

⑦For small-scale reaction, the recommended reaction components are as follows.

DNA	0.1 μg	0.5 μg
XbaI, ADCF	1 U	5 U
10× XbaI Buffer	1 μl	2.5 μl
Total	10 μl[b]	25 μl

b. To avoid evaporation, the incubation time of the 10 μl reaction buffer should not exceed 1 h.

Number of Recognition Sites in DNA

λDNA	ΦX174	pBR322	pUC57	pUC18/19	SV40	M13mp18/19	Adeno2
1	0	0	1	1	0	1	5

Methylation Effects on Digestion

Dam	Dcm	CpG	EcoKI	EcoBI
Some Blocked	No effect	No effect	No effect	No effect