EXact-Cut™ SphI Restriction Endonuclease

Catalog Number: EXNA039
Size: 500 Units

For Research Use Only. Not Intended for Diagnostic or Therapeutic Use.

EXREprotein™

More information: info@exreprotein.com

Product Details

Description	EXact-Cut™ SphI Restriction Endonuclease is engineered for high specificity, reduced star activity, and time saving DNA digestion in 5-15 minutes. To simplify experimental design using multiple restriction enzymes, our entire range of EXact-Cut™ restriction endonucleases are 100% active in our EXact-Cut™ buffer (included) and are optimized for single-tube reactions along with digestion and ligation protocols.
Restriction Enzyme Site	5'…G CATG↓C…3' 3'…C↑GTAC G…5' Isoschizomers: BbuI, PaeI, SpaHI (Isoschizomers may have different methylation sensitivities)
Unit Definition	One unit is defined as the amount of enzyme required to digest 1 µg of λ DNA in 1 hour at 37°C in a total reaction volume of 50 µL.
Recommended Reaction Conditions	1X EXact-Cut™ Buffer Incubate at 37°C Refer to Protocol for reaction setup
Heat Inactivation	1. Incubate at 80°C for 20 minutes 2. Add appropriate volume of 6X Gel Loading Dye, according to the reaction system
Components	EXact-Cut™ SphI (10 Units/µL) 500 Units EXact-Cut™ 10X Buffer 1 mL 6X Gel Loading Dye, Purple 1 mL

Storage and Preparation

Shipping	Shipped on blue ice.
Stability and Storage	Store at -20°C for up to 24 months.

Protocol

Protocol for Rapid DNA Digestion

1. Add the following components on ice in the indicated order:

	Plasmid DNA	PCR Product	Genomic DNA
DNA	≤ 1 µg	≤ 0.2 µg	≤ 5 µg
EXact-Cut™ 10X Buffer	2 µL	3 µL	5 µL
ddH_2O, make up to final volume indicated:	20 µL	30 µL	50 µL
Exact-Cut™ SphI	10 Units	10 Units	30-50 Units

Note: DNA should be free of phenol, chloroform, ethanol, EDTA, detergents or high concentrations of salts. For compatibility with other common buffers, see the chart on page 2.

2. Gently mix or flick the tube to mix (do not vortex), then immediately follow with a quick spin-down in a microcentrifuge.
3. Incubate at 37°C for the indicated sample type: plasmid DNA (15 minutes), PCR product (15-30 minutes), or genomic DNA (30-60 minutes)
4. Optional: inactivate the enzyme at 80°C for 20 minutes and add an appropriate amount of 6X Gel Loading Dye, according to the reaction system.

Protocol for Multiple Digestion of DNA

1. Use 10 Units of each enzyme and scale up to the reaction conditions accordingly.
2. The combined volume of the enzymes in the reaction mixture **should not** exceed **1/10** of the total reaction volume.
3. If the enzymes require different reaction temperatures, start with the enzyme requiring the lowest temperature, followed by the next enzyme(s) and incubate at the higher temperature.
   Note: For total reaction volumes > 20 µL, the incubation time should be increased accordingly in a water bath.

Number of Recognition Sites in DNA

λDNA	ΦX174	pBR322	pUC57	pUC18/19	SV40	M13mp18/19	Adeno2
6	0	1	1	1	2	1	8

Methylation Effects on Digestion

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

Activity in Common Buffers

	EXact-Cut™ Buffer	Takara QuickCut™ Buffer	Thermo Scientific FastDigest Buffer	NEB CutSmart® Buffer
Activity	100%	100%	100%	100%

Application Notes

Functional Test

A 20 µL reaction in EXact-Cut Buffer containing 1 µg of λDNA and 10 Units of EXact-Cut SphI incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.

Digestion-Ligation Test

At the optimal reaction temperature, the DNA was digested using 10 Units of EXact-Cut SphI and the digestion product was recovered. The DNA fragments were ligated using an appropriate amount of T4 DNA Ligase at 22°C. After the ligation product was recovered, it was able to be recut with EXact-Cut SphI.

Non-Specific Endonuclease Activity Test

At the optimal reaction temperature,10 Units of EXact-Cut SphI was incubated in 20 µL reaction volume in EXact-Cut Buffer with 1 µg of supercoiled plasmid DNA for 4 hours. Undigested, supercoiled plasmid DNA was detected using agarose gel electrophoresis.

Blue/White Screening Assay

An appropriate vector containing the *lacZ* gene was digested using 10 Units of EXact-Cut SphI. The digested product was ligated and transformed into *E.coli* cells plated on plates with X-Gal, IPTG and appropriate antibiotic. The successfully ligated *lacZ* gene expresses beta-galactosidase and gives rise to a blue colony, while an interrupted gene (due to degraded DNA end) gives rise to a white colony. Less than 1% white colonies were observed.