

EXact-Cut™ NcoI Restriction Endonuclease

Catalog Number: EXNA024

Size: 500 Units

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

EXREprotein™

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Product Details

Description	EXact-Cut™ NcoI 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'...C↓CATG G...3' 3'...G GTAC↑C...5' Isoschizomers: Bsp191 (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	1 x 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™ NcoI (10 Units/µL) EXact-Cut™ 10X Buffer 6X Gel Loading Dye, Purple	500 Units 1 mL 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 ₂ O, make up to final volume indicated:	20 µL	30 µL	50 µL
EXact-Cut™ NcoI	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.

- Gently mix or flick the tube to mix (do not vortex), then immediately follow with a quick spin-down in a microcentrifuge.
- Incubate at 37°C for the indicated sample type: plasmid DNA (15 minutes), PCR product (15-30 minutes), or genomic DNA (30-60 minutes)
- 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

- Use 10 Units of each enzyme and scale up to the reaction conditions accordingly.
- The combined volume of the enzymes in the reaction mixture **should not** exceed **1/10** of the total reaction volume.
- 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

λ DNA	Φ X174	pBR322	pUC57	pUC18/19	SV40	M13mp18/19	Adeno2
4	0	0	0	0	3	0	20

Methylation Effects on Digestion

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

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 μ L reaction in EXact-Cut Buffer containing 1 μ g of λ DNA and 10 Units of EXact-Cut NcoI 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 NcoI 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 NcoI.

Non-Specific Endonuclease Activity Test

At the optimal reaction temperature, 10 Units of EXact-Cut NcoI was incubated in 20 μ L reaction volume in EXact-Cut Buffer with 1 μ g 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 NcoI. 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.