

NcoI



Catalog: RK21115

Size: 1,000 U / 5,000 U

Concentration: 10,000 U/mL

Components:

NcoI (10,000 U/mL)	RM21617
10X Buffer CutC	RM20106

Product Description

Restriction site:



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.

Storage Conditions:

10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 200 µg/mL BSA, 50% Glycerol, pH 7.4 @ 25°C

Storage Temperature: -20°C

Reaction Conditions:

1X Buffer CutC. Incubate at 37°C.

1X Buffer CutC:

100 mM NaCl, 50 mM Tris-HCl, 10 mM MgCl₂, 100 µg/ml BSA, pH 7.9 @ 25°C

Quick Cut: Yes

This enzyme digests 1 unit substrate in 5-15 minutes under recommended reaction conditions.

Activity in ABclonal Buffer

CutA	CutB	CutC	CutS
100%	100%	100%	100%

Heat Inactivation: 80°C for 20 min

Order: service@abclonal.com

Tech: support@abclonal.com

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Please visit <http://abclonal.com> for a complete listing of recommended products.

Methylation Sensitivity:

dam methylation	Not Sensitive
dcm methylation	Not Sensitive
CpG Methylation	Not Sensitive

Instructions

A "Typical" NcoI Digest:

Composition	Amount
H ₂ O	Up to 50 µL
10x Buffer CutC	5 µL
DNA*	1 µg
NcoI**	0.5-1 µL

- ◆ The substrates are completely digested in 5-15 min incubation at 37°C.
- ◆ *DNA substrates should be free of phenol, chloroform, ethanol, EDTA, detergents, or high concentrations of salt; otherwise, it affects the enzyme activity.
- ◆ **Enzyme amount: 10 units are sufficient; generally, 1 µL is used.

QC Process:

- ◆ Purity is above 95% detected by SDS-PAGE.
- ◆ No exonuclease, nuclease contamination.
- ◆ No residual host genomic DNA detected by PCR.

Optimizing Restriction Endonuclease Reactions

There are several key factors to consider when setting up a restriction endonuclease digest. Using the proper amounts of DNA, enzyme, and buffer components in the correct reaction volume allows you to achieve optimal digestion. By definition, 1 unit of restriction enzyme completely digests 1 µg of substrate DNA in a 50 µL reaction in 60 minutes. This enzyme : DNA : reaction volume ratio can be used as a guide when designing reactions. However, most researchers follow the "typical" reaction conditions listed, where a 5–10 fold overdigestion is recommended to overcome variability in DNA source, quantity, and purity.

A "Typical" Restriction Digest

Restriction Enzyme	10 units are sufficient; generally, 1 μ L is used
DNA	1 μ g
10X ABclonal Buffer	5 μ L (1X)
Total Reaction Volume	50 μ L
Incubation Time	1 hour*
Incubation Temperature	Enzyme dependent

* Can be decreased to 5-15 minutes by using a "Quick Cut" Restriction Enzyme.

Enzyme

- Keep on ice when not in the freezer.
- Should be the last component added to reaction.
- Mix components by pipetting the reaction mixture up and down or by "flicking" the reaction tube. Follow with a quick ("touch") spin-down in a microcentrifuge. Do not vortex the reaction.
- In general, we recommend 5–10 units of enzyme per μ g DNA, and 10–20 units for genomic DNA in a 1 hour digest.

DNA

- Should be free of contaminants such as phenol, chloroform, alcohol, EDTA, detergents, or excessive salts. Extra wash steps during purification are recommended.
- Methylation of DNA can inhibit digestion with certain enzymes.

Buffer

- Use at a 1X concentration
- Supplement with SAM (S-Adenosyl methionine) to the recommended concentration if required.

Reaction Volume

- A 50 μ L reaction volume is recommended for digestion of 1 μ g of substrate.
- Enzyme volume should not exceed 10% of the total reaction volume to prevent star activity due to excess glycerol.
- Additives in the restriction enzyme storage buffer (e.g.,

glycerol, salt), as well as contaminants found in the substrate solution (e.g., salt, EDTA, or alcohol), can be problematic in smaller reaction volumes. The following guidelines can be used for techniques that require smaller reaction volumes.

Reaction System	Enzyme Amount*	DNA	10X ABclonal Buffer
10 μ L**	1 U	0.1 μ g	1 μ L
25 μ L	5 U	0.5 μ g	2.5 μ L
50 μ L	10 U	1 μ g	5 μ L

* Restriction Enzymes should be diluted when smaller amounts are needed. ** 10 μ L rxns should not be incubated for longer than 1 hour to avoid evaporation.

Incubation Time

- Incubation time is typically 1 hour.
- Can often be decreased by using an excess of enzyme, or by using one of our "Quick Cut" restriction enzymes.
- It is possible, with many enzymes, to use fewer units and digest for up to 16 hours.

Stopping a Reaction

If no further manipulation of DNA is required:

- Terminate with a stop solution (10 μ L per 50 μ L rxn) [1x: 2.5% FicolI-400, 10 mM EDTA, 3.3 mM Tris-HCl, 0.08% SDS, 0.02% Tartrazine, 0.001% Xylene Cyanol FF, pH 8.0 @ 25°C].

When further manipulation of DNA is required:

- Heat inactivation can be used.
- Remove enzyme by using a spin column or phenol/chloroform extraction

Control Reactions

If you are having difficulty cleaving your DNA substrate, we recommend the following control reactions:

- Control DNA (DNA with multiple known sites for the enzyme, e.g., lambda or pUC19 DNA) with restriction enzyme to test enzyme viability.
- If the control DNA is cleaved and the experimental DNA resists cleavage, the two DNAs can be mixed to determine if an inhibitor is present in the experimental sample. If an inhibitor (often salt, EDTA, or phenol) is present, the control DNA will not cut after mixing.