NotI

Cat. No. : RK21103

Product Components

Components	Component Number	Concentration	500 U	2,500 U
NotI	RM21600	20,000 U/mL	25 μL	125 μL
10X Buffer CutS	RM20103	10X	1.25 mL	1.25 mL

Product Description

Restriction Site

5'...G C G G C C G C...3'

3'...C G C C G G C G...5'

Unit Definition

One unit is defined as the amount of enzyme required to digest 1 μg of pBC4 DNA in 1 hour at 37°C in a total reaction volume of 50 μL.

Storage

-20°C

Reaction Conditions

1X Buffer CutS, incubate at 37°C.

1X Buffer CutS

50 mM KAc, 20 mM Tris-HAc, 10 mM $MgAc_2$,

100 μg/mL rHSA, pH 7.9 @ 25°C

Quick Cut

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

Heat Inactivation

65°C for 20 min.

Methylation Sensitivity

dam methylation	dcm methylation	CpG Methylation
not sensitive	not sensitive	Blocked

Instructions

Recommended Protocol for Digestion

Components	50 μL
ddH_2O	Up to 50 μL
10X Buffer CutS	5 μL
DNA*	1 μg
NotI	1 μL

* Note: DNA substrates should be free of phenol, chloroform, ethanol, EDTA, detergents or high concentrations of salt, otherwise it will affect the enzyme activity.

- The substrates are completely digested in 5-15 min incubate at 37°C.

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 will allow you to achieve optimal digestion. 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 is sufficient, generally 1 μL is used
DNA	1 μg
10X ABclonal Buffer	5 μL (1X)
Total Reaction Volume	50 μL
Incubation Time	1 hr*
Incubation Temperature	Enzyme dependent

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

1. Enzyme

- In general, we recommend 5 - 10 units of enzyme per µg DNA, and 10–20 units for genomic DNA in a 1 hour digest.

2. DNA

- Methylation of DNA can inhibit digestion with certain enzymes.

3. Buffer

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

4. Reaction Volume

- A 50 µL reaction volume is recommended for digestion of 1 µg 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 µL**	1 U	0.1 µg	1 µL
25 µL	5 U	0.5 µg	2.5 µL
50 µL	10 U	1 µg	5 µL

* Note: Restriction Enzymes should be diluted when smaller amounts are needed.

** Note: 10 µL rxns should not be incubated for longer than 1 hour to avoid evaporation.

5. 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.

6. Stopping a Reaction

If no further manipulation of DNA is required:

- Terminate with a stop solution (10 µL per 50 µL rxn) [1x: 2.5% Ficoll-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.

7. 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.