



Restriction Enzyme Not I

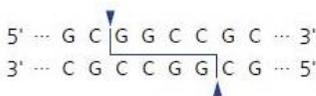


Cat.# FG-NotI	Size 500 units	Conc. 10 units/μl
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Store at -20°C

Supplied with: FastGene® 10X Buffer III (FG-REB3)
FastGene® 10X FastCut Buffer (FG-REBHF)
6X DNA Loading Buffer
Sterile water

Recognition site



For Research Use Only. Not for use in diagnostic procedures.



Dilution buffer

FastGene® Diluent C

Heat Inactivation

FastGene® Not I can be inactivated at 65°C for 20 min.

Methylation sensitivity

dam methylation: Not sensitive
dcm methylation: Not sensitive
CpG methylation: Sensitive

Prolonged incubation

A minimum amount of enzyme required to digest 1 μg substrate DNA for 16 hr; 0.25 U.

Relative activity in FastGene® Buffers

FastGene® Buffer I:	0%
FastGene® Buffer II:	50%
FastGene® Buffer III:	100%
FastGene® Buffer IV:	0%
FastGene® FastCut Buffer:	100%

Note

Cleavage of mammalian genomic DNA is blocked by CpG methylation. Supercoiled plasmids may require up to 5-fold more enzyme for complete digestion than linear DNA. Cutting of DNA with less than 8 bases on either side of the recognition site is poor. It is suitable to generate large DNA fragments due to the rare occurrence of the recognition site.

Source: *Nocardia otitidis-caviarum*

Reaction conditions

1X FastGene® 10X Buffer III, 37°C
1X FastGene® 10X FastCut Buffer , 37°C

FastGene® FastCut Buffer

FastGene® restriction enzyme can cut substrate DNA in 5-15 min with FastGene® FastCut Buffer.

1X FastGene® 10X Buffer III

50 mM Tris-HCl (pH 7.9 at 25°C)
100 mM NaCl
10 mM MgCl₂
100 μg/ml BSA

Unit definition

Half unit is defined as the amount of enzyme required for >50% digestion of pSK M2 at 37°C for 1 hr in 50 μl reaction mixtures.

Quality control

- Unit definition assay
- Overdigestion assay
- Endonuclease assay
- Extreme pure assay

Standard reaction condition

- Normal protocol

Component	Final Conc.	Volume
Substrate DNA	1 μg	X μl
10X FastGene® Buffer III	1 X	5 μl
Not I	10 unit	1 μl
Sterile water		up to 50 μl

→ Incubate at 37°C for 1 hr

- Fast protocol

Component	Final Conc.	Volume
Substrate DNA	1 μg	X μl
10X FastGene® FastCut Buffer	1 X	5 μl
Not I	10 unit	1 μl
Sterile water		up to 50 μl

→ Incubate at 37°C for 15 min

※We recommend 5-10 units of enzyme per μg DNA and 10-20 units for genomic DNA in a 1 h digest.