

Product Specification

Product Name: SpeedyCut *NotI* Restriction Enzyme
Product Code: SGRE02800

Product Description:

NotI is categorized as a SpeedyCut series Restriction Enzymes (RE). It is a subset of RE that are capable of cutting DNA within 5 – 15 minutes. All SpeedyCut RE are compatible with the universal SpeedyOne buffer, this universal buffer allows for combination of different enzymes within a standardized reaction system without the need for multiple rounds of single enzyme cuts. They are convenient, stable, fast and can accurately cut DNA.

NotI is a type of Type II class RE. The SpeedyCut series RE undergoes strict quality control processes to ensure its reliability for rapid digestion of plasmid DNA, genomic DNA and PCR products.

***NotI* Recognition Site**

5'...-GC↓GGCCGC-...3'
3'...-CGCCGG↑CG-...5'

Product Content(s)

SpeedyCut *NotI* RE 50µl
10X SpeedyOne buffer 1ml

Storage: -20°C

Note: At 37°C, in a 20µl reaction system, 1µl enzyme can completely digest 1µg N85 DNA in 15 minutes. If there's a difference in the optimal temperatures of the chosen enzymes, consider starting the digestion reaction with the enzyme having the lower optimal temperature, and then subsequently add the enzyme with the higher optimal temperature.

Procedures:

Recommended DNA digestion process

- a. Begin by preparing the reaction setup, ensuring that all required reagents are kept on ice

Reagents	Volume
10X SpeedyOne Buffer	2µl
DNA (0.5~1µg/µl)	1µl
SpeedyCut <i>NotI</i>	0.5~1µl
Nuclease – free water	16µl
Total	20µl

Note: The volume of all enzyme added should not exceed 10% of the total volume to prevent an excess of glycerol in the enzyme, which may lead to star activity.

PCR Product Directly After Amplification

- a. Begin by preparing the reaction setup, ensuring that all required reagents are kept on ice

Reagents	Volume
10X SpeedyOne Buffer	2µl
DNA (0.5~1µg/µl)	10µl
SpeedyCut <i>NotI</i>	1~2µl
Nuclease – free water	7µl
Total	20µl

- b. Pipette all reagents into a microcentrifuge tube (according to the tables above) and gently resuspend to ensure it is homogenized (Do not Vortex). Immediately centrifuge the tubes to ensure no reagents are left on the side of the tube.
- c. Incubate at 37°C for 15 minutes for plasmid DNA, 15 – 30 minutes for PCR products or 30 – 60 minutes for genomic DNA.
- d. Incubate at 80 °C for 20 minutes to inactivate enzyme and stop the reaction (optional). Alternatively, purification method like spin column or phenol / chloroform extraction could be used to stop the reaction.

Enzyme properties

1. Methylation Effects

- a. Dam: Never overlap – no effects
 b. Dcm: Never overlap – no effects
 c. CpG: Overlap – digestion is hindered

2. Heat Inactivation

- a. Heat inactivated at 80 °C for 20 minutes

3. Number of recognition sites in DNA

- a. λDNA – 0
 b. φX174 - 0
 c. M13mp18/19 - 0
 d. pBR322 – 0
 e. pUC57 – 0
 f. pUC18/19 – 0

Isochizomers

- a. *CciNI*

*Isochizomers may have different sensitivity to different methylation modification

Note:

This product is designed for research use only. Not to be used on humans or animals' diagnosis and other in vivo experiments.