Nhe Ⅰ

Instruction for Use

Cat. No./Spec.: E1030-A/50 rxns, E1030-B/100 rxns

Recognition site:

5' G↓CTAGC 3'

3' CGATC↑G 5'

Product Description

The Nhe Ⅰ restriction endonuclease recognizes the G^CTAGC site and achieves optimal cutting efficiency within 5 – 15 minutes at 37 ° C using the universal buffer. Isoschizomers: AsuNH Ⅰ, Bmt Ⅰ.

GDSBio restriction endonucleases exhibit 100% activity in both Digest and Green reaction buffers.

The universal Digest buffer allows for rapid single-enzyme, double-enzyme, or multiple-enzyme digestion of DNA within 5–15 minutes, eliminating the need for buffer exchange or subsequent DNA purification steps. DNA-modifying enzymes (such as the Klenow fragment, T4 DNA ligase, calf intestinal alkaline phosphatase, and T4 DNA polymerase) maintain 100% activity in the buffer. Consequently, enzymes used in downstream applications can be directly added to the reaction mixture. Shorter incubation times and the superior composition of the universal Digest buffer eliminate the star activity effect.

The Green buffer includes one density reagent and two tracking dyes for direct loading of the digestion products onto a gel.

Components

Component	E1030-A	E1030-B
Nhe Ⅰ	50 μL	100 μL
10X Digest Buffer	1 mL	1 mL
10X Green Buffer	1 mL	1 mL

Storage Condition & Shelf Life

Store at -20°C.

Features

- All GDSBio restriction endonucleases exhibit 100% activity in the universal buffer.

- 100% buffer compatibility with downstream applications.

- Enzymatic digestion can be completed within 5 – 15 minutes.

- Direct loading onto a gel.

- No star activity.

Scope of Application

- Molecular cloning

- Restriction site mapping

- Genotyping

- Southern blotting

- Restriction fragment length polymorphism (RFLP)

- SNP (Single Nucleotide Polymorphism) analysis

Recommended Reaction Conditions

• 1X Digest Buffer or 1X Green Buffer

• Incubate at 37°C.

• Maximum amounts for enzymatic digestion with 1 μL Nhe Ⅰ:

– 1 μg lambda DNA in 5 minutes

– 1 μg plasmid DNA in 15 minutes

– 0.2 μg PCR product in 5 minutes

– 1 μg genomic DNA in 10 minutes, or 5 μg genomic DNA in 30 minutes

Inactivation

Incubation at 65°C for 5 min.

Methylation Effects on Digestion

Dam: never overlaps – no effect.

Dcm: never overlaps – no effect.

CpG: may overlap – cleavage impaired.

EcoKI: never overlaps – no effect.

EcoBI: never overlaps – no effect.

Number of Recognition Sites in DNA

λDNA	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
1	0	1	0	0	0	0

Protocol

Fast Digestion of Different DNA

① *Prepare the reaction system at room temperature in the following order:*

Component	Plasmid DNA	PCR product	Genomic DNA
Nuclease-free Water	15 μL	17 μL	30 μL
10X Digest Buffer or10X Green Buffer	2 μL	2 μL	5 μL
DNA	2 μL (up to 1 μg)	10 μL (~0.2 μg)	10 μL (5 μg)
enzyme	1 μL	1 μL	5 μL
Total volume	20 μL	30 μL	50 μL

② *Gently mix and briefly centrifuge.*

③ *Incubate at 37°C for 5 minutes (PCR products and genomic DNA), or for 15 minutes (plasmid DNA).*

Optional: Heat at 65°C for 5 minutes to inactivate the enzyme.

Double and Multiple Digestion of DNA

- The total volume of enzyme in the reaction mixture should not exceed 1/10 of the total reaction volume.

- Use 1 μL of each enzyme and scale up the reaction conditions accordingly.

- If the enzymes require different reaction temperatures, start with the enzyme that requires the lower temperature, then add the second enzyme and incubate at the higher temperature.

Scaling up Plasmid DNA Digestion Reaction

Component	20-μL rxn	20-μL rxn	30-μL rxn	40-μL rxn	50-μL rxn
DNA	1 μg	2 μg	3 μg	4 μg	5 μg
enzyme	1 μL	2 μL	3 μL	4 μL	5 μL
10X Digest Buffer or 10X Green Buffer	2 μL	2 μL	3 μL	4 μL	5 μL
Total volume	20 μL	20 μL	30 μL	40 μL	50 μL

Note: If the total reaction volume exceeds 20 μL, increase the incubation time by 3-5 minutes. Use a water bath for temperature control; an air incubator is not recommended as heat transfer to the reaction mixture is slow.

This product is for research use only.