NheI

5'-G C T A G C -3'
3'-C G A T C G -5'

Cat. No.	Size
E2294-01	500 units
E2294-02	2 500 units

Reaction Temperature: 37°C

Inactivation Temperature (20 min): 65°C

Prototype: NheI

Source: *Neisseria mucosa heidelbergensis*
Note 1: Recombinant. Purified from an *E.coli* strain carrying the NheI gene from *Neisseria mucosa heidelbergensis.*

Package Contents:

- **NheI**
- **10x Reaction Buffer NheI**
- **BSA [100x]**
  Added as separate component to prevent reaction buffer precipitation.

Storage Conditions: Store at -20°C

Double Digestion – Buffer Compatibility:

Buffer	% Rel. Activity
Low	50
Medium	0
High	0
Acet	50

Recommended Buffer: NheI
(or compatible third party buffers)

Restriction Enzyme Buffer Compatibility:
Both, enzyme and buffers are fully compatible to restrictases and buffer systems from other manufacturers and can be used along in double digestions. To obtain best results, consult the corresponding manuals of all involved products.

DNA Methylation:
No inhibition: dam, dcm, EcoKI
Potential inhibition: CpG

Standard Reaction Protocol:

Mix the following reaction components:
1-2 µg pure DNA or 10 µl PCR product (=~0.1-2 µg DNA)
2 µl 10x Buffer NheI
0.2 µl BSA [100x]
1-2 U NheI (use 1 U / µg DNA, < 10 % React. Volume!)
Tips: Add enzyme as last component. Mix components well before adding enzyme. After enzyme addition, mix gently by pipetting. Do not vortex. High (excess) amounts of enzyme can greatly speed up the reaction.
@ 20 µl H_2O, nuclease free

Incubate for 1 h at 37°C
To obtain complete digestion of high molecular weight DNA, (e.g. plant genomic DNA), add excess amounts of enzyme and prolong the incubation time.

Stop reaction by alternatively
(a) Addition of 0.8 µl EDTA pH 8.0 [0.5 M], final 20 mM *or*
(b) Heat Inactivation
20 min at 65°C *or*
(c) Spin Column DNA Purification
(e.g. EURx PCR/DNA CleanUp Kit, Cat.No. E3520) *or*
(d) Gel Electrophoresis and Single Band Excision
(e.g. EURx AgaroseOut DNA Kit, Cat.No. E3540) *or*
(e) Phenol-Chloroform Extraction or Ethanol Precipitation.

Non-optimal buffer conditions:

To compensate for the lack of enzyme activity, increase the amount of enzyme and / or reaction time accordingly. The following values may serve as orientation:

1. *Enzyme amount*: Instead of 1 U enzyme, use ~4 U of enzyme in buffers providing 25 % rel. activity, ~2 U in 50 %, ~1.5 U in 75 % or ~1 U in 100 %, respectively.
2. *Reaction time*: Increase by ~1.3-fold (75 % rel. activity), ~2-fold (50 %) or ~4-fold (25 %).

Unit Definition:

One unit is the amount of enzyme required to completely digest 1 µg of Ad-2 DNA in 1 hr in a total reaction volume of 20 µl. Enzyme activity was determined in the recommended reaction buffer.

Note 2: To avoid star activity it is not recommended:
- **to use more than 100 units per reaction;**
- **to incubate over 4 hours.**

Note 3: Salt concentration above 50 mM inhibits NheI activity.

Reaction Buffer:

1 x NheI Buffer: 20 mM Tris-HCl (pH 7.4 at 25°C), 5 mM $MgCl_2$, 50 mM KCl.
To be supplemented with 100 µg/ml bovine serum albumin.

Storage Buffer:

10 mM Tris-HCl (pH 7.5 at 25°C), 1 mM dithiothreitol, 250 mM NaCl, 0.1 mM EDTA, 0.1 % Triton X-100, 500 µg/ml bovine serum albumin and 50 % (v/v) glycerol.

Quality Control:

All preparations are assayed for contaminating endonuclease, 3'-exonuclease, and nonspecific single- and double-stranded DNase activities.