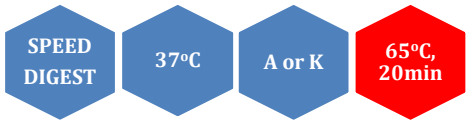
Nhe I



5’ ···G▼CTAGC···3’
3’ ···CGATC▲G···5’

NheI is a restriction enzyme purified from *Neisseria mucosa heildelbergensis* (ATCC 25999).

Catalogue No — *146-1, 1000 U*
146-2, 3x1000 U

Concentration — *10-12u/µl and 40-60u/µl**

**Add an H to cat.# to order the high concentration*

Reagents supplied: 10x A and 10x K buffer

Unit substrate: Lambda DNA (*Hind*III digest).

Unit calculation assay conditions: 50 mM potassium acetate, 20 mM Tris-acetate (pH 7.9 @ 25°C), 10 mM magnesium acetate, 1 mM dithiothreitol, 100 µg/ml BSA**.** Incubate at 37°C.

Absence of contaminants: 80 units of *Nhe*I do not produce any unspecific cleavage products after 16 hrs incubation with 1 µg of λ DNA/Hind III digest at 37°C. After 100-fold overdigestion with *Nhe*I, greater than 98% of the DNA fragments can be ligated and recut.

Storage buffer: 200 mM NaCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM dithiothreitol, 0.15% Triton X-100, 200 µg/ml BSA and 50% glycerol. Store at -20°C.

Heat inactivation: 65°C for 20 minutes..

Methylation Sensitivity:
dam methylation: Not sensitive
dcm methylation: Not sensitive
CpG methylation: Blocked by some combinations of overlapping

Note: Activity inhibited by salt concentrations >100mM. Cleaves to leave a 5’ CTAG extension which can be efficiently ligated to DNA fragments generated by *Avr*II, *Spe*I, or *Xba*I.

Star activity: Low salt, high glycerol (>5%) concentrations, pH >8.0 or large excess of the enzyme may result in star activity.

Percent Activity in MINOTECH Buffers

L	M	H	SH	**A**	**K**
100	50-75	0-20	<10	**100**	**100**

General reaction mixture:

10U NheI	1µl
10x A or K buffer *	2µl
DNA substrate	<1µg
Sterile ultrapure water	Up to 20 µl

Incubate for 15 min at 37°C

**In the case of A buffer we recommend the addition of BSA to a final concentration of 100 µg/ml.*

Frequency of Cutting

λ	Ad-2	Φx174	pUC18	M13mp18	pBR322
1	4	0	0	0	1

Lambda DNA 0.7 % agarose

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