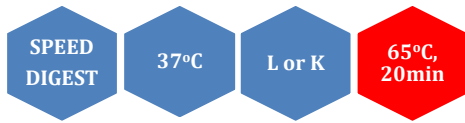


Nae I



5' ...GCC▼GGC...3'
3' ...CGG▲CCG...5'

Nae I is a restriction enzyme purified from *Streptomyces* species.

Catalogue No 122-1, 500 U
 122-2, 3x500 U

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

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

Reagents supplied: 10x L and 10x K buffer

Unit substrate: pBR322 DNA.

Unit calculation assay conditions: 10 mM Tris-HCl (pH 7.9 @ 25°C), 10 mM MgCl₂, 1 mM dithiothreitol, 100 μg/ml BSA. Incubate at 37°C.

Absence of contaminants: 50 units of Nae I do not produce any unspecific cleavage products after 16 hrs incubation with 1 μg of pBR322 at 37°C. After 10-fold overdigestion with Nae I, greater than 80% of the DNA fragments can be ligated and recut with this enzyme.

Storage buffer: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 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

Note: Nae I exhibits site preferences. pBR322 contains four Nae I recognition sequences. Two of these sites are readily cleaved, one is cleaved moderately slowly, and the fourth is cleaved 50-fold more slowly.

Percent Activity in MINOTECH Buffers

L	M	H	SH	A	K
100	25-50	25	<10	50	100

General reaction mixture:

10U Nae I	1μl
10x L or K buffer *	2μl
DNA substrate	<1μg
Sterile ultrapure water	Up to 20 μl
<i>Incubate for 15 min at 37°C</i>	

*In the case of L 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	13	0	0	1	4



pBR322 0.7 % agarose