NarI

5'-G G C G C C-3'
3'-C C G C G G-5'

Cat. No.	Size
E2291-01	200 units
E2291-02	1 000 units

Reaction Temperature: 37°C

Inactivation Temperature (20 min): 65°C

Prototype: NarI

Source: *Nocardia argentinensis*

Package Contents:

- **NarI**
- **10x Reaction Buffer Low**
- **BSA [100x]**
  Added as separate component to prevent reaction buffer precipitation.
- **Dilution Buffer # 1**
  Added for enzymes exceeding 10 U/µl in concentration. High protein concentration warrants optimal stability during prolonged storage. Use dilution buffer to prepare short term working stocks (5-10 U/µl, non-freezing at -20°C).

Storage Conditions: Store at -20°C

Double Digestion – Buffer Compatibility:

Buffer	% Rel. Activity
Low	**100**
Medium	50
High	50
Acet	75

Note: Some NarI sites are cut very slowly for example on pBR322 the NarI site at 548 bp.

Recommended Buffer: Low
(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, EcoKI
Potential inhibition: dcm
Inhibition (Blocked): CpG

Standard Reaction Protocol:

Mix the following reaction components:
1-2 µg pure DNA or 10 µl PCR product (=~0.1-2 µg DNA)
5 µl 10x Buffer Low
0.5 µl BSA [100x]
1-2 U NarI (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.
@ 50 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 2.1 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 50 µl. Enzyme activity was determined in the recommended reaction buffer.

Reaction Buffer:

1 x Low Buffer: 10 mM Tris-HCl (pH 7.5 at 22°C), 10 mM $MgCl_2$, 1 mM dithiothreitol.
To be supplemented with 100 µg/ml bovine serum albumin.

Storage Buffer:

10 mM Tris-HCl (pH 7.8 at 22°C), 50 mM KCl, 1 mM dithiothreitol, 0.1 mM EDTA, 500 µg/ml bovine serum albumin and 50 % (v/v) glycerol.

Quality Control:

All preparations are assayed for contaminating endonuclease, 3'-exonuclease, 5'-exonuclease/5'-phosphatase, as well as non-specific single- and double-stranded DNase activities.