NotI

Not I
Restriction Endonuclease

Recognition Sequence:

5`-G C G G C C G C-3`
3`-C G C C G G C G-5`

Cat. No.	Size
E2295-01	500 units
E2295-02	2 500 units

Reaction Temperature: 37°C

Inactivation Temperature (20 min): 65°C

Prototype: NotI

Source: *Nocardia otitidis-caviarum*

Package Contents:
- NotI
- 10x Reaction Buffer High
- BSA [100x]
  Added as separate component to prevent reaction buffer precipitation.
- Dilution Buffer # 3
  Added only for enzymes exceeding 10 U/µl in concentration. Use dilution buffer to dilute working stocks of enzyme to a customary concentration of 5 to 10 U/µl. Diluted enzyme stocks will not freeze during storage at -20°C.

Storage Conditions: Store at -20°C

Double Digestion – Buffer Compatibility:

Buffer	% Relative Activity
Low	0
Medium	75
High	**100**
Acet	25

Recommended Buffer: High
(or compatible third party buffers)

DNA Methylation:
No inhibition: dam, dcm, EcoKI
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 High
0.5 µl BSA [100x]
1-2 U NotI (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, DNA and DNase 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:
- *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.
- *Reaction time:* Increase by ~1.3-fold (75 % rel. activity), ~2 fold (50 %) or ~4 fold (25 %), respectively.

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 High Buffer: 50 mM Tris-HCl (pH 7.5 at 37°C), 10 mM $MgCl_2$, 100 mM NaCl, 1 mM dithiothreitol.
To be supplemented with 100 µg/ml bovine serum albumin.

Reaction 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.

Storage Buffer:

20 mM Tris-HCl (pH 7.5 at 22°C), 100 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol, 0.01 % [v/v] Triton™X-100, 500 µg/ml bovine serum albumin and 50 % [v/v] glycerol.

Quality Control:

All preparations are assayed for nicking activity, for contaminating endonuclease, 3'-exonuclease, 5'-exonuclease/5'-phosphatase, as well as nonspecific single- and double-stranded DNase activities. Ligation / recut assay verified proper enzyme performance.

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