



NotI RS*

- reduced star activity -

NotI red. star

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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
E2296-01	500 units
E2296-02	2 500 units

Reaction Temperature: 37°C

Inactivation Temperature (20 min): 65°C

Prototype: NotI

Source: Gene from *Nocardia otitidis-caviarum*
Recombinant, purified from an *E. coli* strain carrying the NotI gene from *Nocardia otitidis-caviarum*

Package Contents:

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

Storage Conditions: Store at -20°C

Double Digestion – Buffer Compatibility:

ONE Buffer is compatible with most EURx restriction enzymes.

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 ONE
- 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 H₂O, 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 pBC4 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 ONE Buffer

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 4°C), 200 mM NaCl, 0.1 mM EDTA, 1 mM dithiothreitol, 0.15 % [v/v] Tergitol™ TMN, 200 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.