EURx® MOLECULAR BIOLOGY PRODUCTS

NotI RS*

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: Recombinant. Purified from an *E.coli* strain carrying the cloned NotI gene from *Nocardia otitidis-caviarum*.

***Reduced star activity**

Package Contents:

- **NotI**
- **10 x ONE Buffer**
- **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.

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, dcm, EcoKI

Inhibition (Blocked): CpG

Standard Reaction Protocol (for 50 µl volume):

Mix the following reaction components:

1-2 µg pure DNA or 10 µl PCR product (=~0.1-2 µg DNA)

5 µl 10 x ONE Buffer

0.5 µl BSA [100x]

1-2 U NotI (use 1 U per 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.

add sterile H_2O to 50 µl final volume

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 Clean-Up Kit, Cat.No. E3520) *or*

(d) Gel Electrophoresis and Single Band Excision

(e.g. EURx Agarose-Out 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 pBC4 DNA in 1 hr. Total reaction volume is 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.

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 contaminating endonuclease, 3'-exonuclease, 5'-exonuclease/5'-phosphatase, as well as for nonspecific single- and double-stranded DNase activities. Ligation / recut assays passed successfully.

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