

DATA SHEET

**Not I**

Isoschizomers: CciN I

Cat. Nº.	Amount
<input type="checkbox"/> ENZ-110XS	300 units
<input type="checkbox"/> ENZ-110S	2 x 300 units (600 units)
<input type="checkbox"/> ENZ-110M	5 x 300 units (1.500 units)
<input type="checkbox"/> ENZ-110L	10 x 300 units (3.000 units)



Unit Definition: One unit is the amount of enzyme required to completely digest 1 µg of Adenovirus-2 DNA (7 sites) in 1 hour in a total reaction volume of 50 µl. Enzyme activity was determined in the recommended reaction buffer.

Source:*Nocardia otitidis-caviarum***For in vitro use only!****Shipping:**

Shipped on blue ice

Storage Conditions:

Store at -20 °C

Additional Storage Conditions:

Avoid freeze/thaw cycles.

Shelf Life:

12 months

Form:

Liquid (Supplied in 10 mM Tris-HCl pH 7.4, 500 mM KCl, 0.1mM EDTA, 1 mM DTT, 500 µg/ml BSA, 0.1% Triton X-100 and 50 % [v/v] glycerol).

Concentration:

10 units/µL

Supplied with:

10X Universal Buffer (UB)

Recommended 50 µL assay:

Components	50 µL rxn
10X Universal Buffer (UB)	5 µl
Pure DNA ¹ or PCR Product ²	1 µg
Enzyme	5 units (0.5 µL)
PCR Grade water	fill up to 50 µL

¹ Supercoiled or high molecular weight DNA (e.g. plant genomic DNA) may require longer incubation time or higher amount of enzyme.

² Some enzymes may require additional DNA bases flanking the restriction site for complete digestion.

Double Digestion - Buffer Compatibility

- B1 - <10% Relative Activity
- B2 - 25-50% Relative Activity
- B3 - 75-100% Relative Activity
- B4 - 75% Relative Activity
- B5 - 50% Relative Activity
- 1x UB - 100 % Relative Activity (recommended)

Please note that the optimum digestion condition for this enzyme is 1x UB. Within the Universal Buffer (UB) system, the most majority of our enzymes display 100% Relative Activity in 1x UB and only few either in 0.5x or 2x UB. If optimum condition for second enzyme is different than the recommended for the first enzyme, we suggest carrying out first the restriction at the higher recommended concentration of UB and dilute the reaction volume to the adequate UB concentration for further proceeding with the second restriction.

Protocol:

- The enzyme should not exceed 10 % of total reaction volume.
- Add enzyme as last component. Mix components well before adding enzyme. After enzyme addition, mix gently by pipetting. Do not vortex.
- Incubate 60 min at 37 °C.
- Stop reaction by alternatively:
 - Addition of 2.1 µl EDTA pH 8.0 [0.5 M], final 20 mM
 - Heat Inactivation (20 min. at 80 °C)
 - Spin Column DNA Purification (e.g. PCR Purification Kit, Cat. #DPK-106)
 - Gel Electrophoresis and Single Band Excision (e.g. Agarose Gel Extraction Kit, Cat.#DPK-105)
 - Phenol-Chloroform Extraction or Ethanol Precipitation.

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Reaction Enzymes Buffer Guide:

Buffer 1	10X B1	100 mM Tris-HCl (pH 7.9, 25 °C) 100 mM MgCl ₂ 1000 µg/mL BSA
Buffer 2	10X B2	100 mM Tris-HCl (pH 7.9, 25 °C) 100 mM MgCl ₂ 500 mM NaCl 1000 µg/mL BSA
Buffer 3	10X B3	500 mM Tris-HCl (pH 7.9, 25 °C) 100 mM MgCl ₂ 1000 mM NaCl 1000 µg/mL BSA
Buffer 4	10X B4	100 mM Tris-HCl (pH 7.9, 25 °C) 100 mM MgCl ₂ 1500 mM NaCl 1000 µg/mL BSA
Buffer 5	10X B5	200 mM Tris-acetate (pH 7.9, 25 °C) 100 mM Mg-acetate 500 mM K-acetate 1000 µg/mL BSA

Reaction Buffer Compatibility:

Our restriction enzymes 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.

Ligation and recutting:

After 25-fold overdigestion with Not I, >98% of the DNA fragments can be ligated and recut with this enzyme.

Note:

Supercoiled plasmids may require up to 5-fold more Not I for complete digestion than linear DNA.

DNA Methylation:

No Inhibition: dam, dcm

Inhibition (Blocked): CpG

Quality Control:

All preparations are assayed for contaminating endonuclease, 3'-exonuclease, 5'-exonuclease/ 5'-phosphatase, as well as nonspecific single- and doublestranded DNase activities.