

Not I  
5`...GCGGCCGC...3`  
3`...CGCCGGCG...5`

Source: *Nocardia otitidis-caviarum*.

---

Cat.-No.	Size	Conc.
EN-124	10,000 units	10 u/μl

**Buffer supplied:** 10x Not I Buffer and 10x BSA.

**Substrate for unit definition:**  
Adenovirus-2 DNA (7 sites).

**Reaction conditions:**  
100 mM NaCl, 50 mM Tris-HCl (pH 7.9), 5 mM MgCl<sub>2</sub>, 1 mM DTT, 100 μg/ml BSA. Incubate at **37°C**.

**Storage buffer:**  
500 mM KCl, 10 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 0.1% Triton X-100, 500 μg/ml BSA, and 50% glycerol. Store at -20°C.

**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.

**Heat inactivation:** 65°C for 20 minutes.