

# Not I

## 1 Contents

Component	HY-KE7028-50T
Not I	50 µL
10× Buffer	1 mL
10× Color Buffer	1 mL

## 2 Introduction

Not I is a restriction enzyme for rapid DNA digestion, including plasmid, genomic DNA as well as PCR products. Isoschizomers: CciN I.

Not I is 100% active in 10× Buffer and 10× Color Buffer. 10× Color Buffer includes a density reagent along with red and yellow tracking dyes that allow for direct loading of the reaction mixtures on a gel. The red dye of the Color Buffer migrates with 2500 bp DNA fragments in a 1% agarose gel and the yellow dye of the Color Buffer migrates faster than 10 bp DNA fragments in a 1% agarose gel.

### Cleavage site



## 3 General Protocol

### 1. Fast Digestion of Different DNA

1.1 Combine the following reaction components on ice in the order indicated:

	Plasmid DNA	PCR product	Genomic DNA
ddH <sub>2</sub> O	15 µL	16 µL	30 µL
10× Buffer or 10× Color Buffer	2 µL	3 µL	5 µL
DNA	2 µL (up to 1 µg)	10 µL (~ 0.2 µg)	10 µL (5 µg)
Not I	1 µL	1 µL	5 µL
Total	20 µL	30 µL	50 µL

Note: When PCR product will be used for cloning, it is recommended to purify PCR product prior digestion.

1.2 Mix gently and spin down.

1.3 Incubate at 37°C for 15 min (Plasmid DNA), or for 15-30 min (PCR product), or for 30-60 min (Genomic DNA).

1.4 (Optional) Inactivate the enzyme by heating for 20 min at 80°C.

1.5 If 10× Color Buffer was used in the reaction, load an aliquot of the reaction mixture directly on a gel.

## 2. Scaling up Plasmid DNA Digestion Reaction

DNA	1 µg	2 µg	3 µg	4 µg	5 µg
Not I	1 µL	2 µL	3 µL	4 µL	5 µL
10× Buffer or 10× Color Buffer	2 µL	2 µL	3 µL	4 µL	5 µL
Total	20 µL	20 µL	30 µL	40 µL	50 µL

Note: Increase the incubation time by 3-5 min if the total reaction volume exceeds 20 µL. It is recommended to use heat block or water thermostat.

## 4 Number of Recognition Sites in DNA

λDNA	ΦX174	pBR322	pUC57	pUC18/19	SV40	M13mp18/19	Adeno2
0	0	0	0	0	0	0	7

## 5 Methylation Effects on Digestion

Dam	Dcm	CpG	EcoKI	EcoBI
no effect	no effect	blocked	no effect	no effect

## 6 Storage

-20°C, 2 years.

## 7 Precautions

1. The combined volume of the enzymes in the reaction mixture should not exceed 1/10 of the total reaction volume.
2. Use 1 µL of each enzyme and scale up the reaction conditions appropriately.
3. If the enzymes require different reaction temperatures, start with the enzyme that requires a lower temperature, then add the second enzyme and incubate at the higher temperature.
4. This product is for R&D use only, not for drug, household, or other uses.
5. For your safety and health, please wear a lab coat and disposable gloves to operate.