

# Hind III

## 1 Contents

| Component        | HY-KE7018-500T |
|------------------|----------------|
| Hind III         | 500 µL         |
| 10× Buffer       | 1 mL × 3       |
| 10× Color Buffer | 1 mL × 3       |

## 2 Introduction

Hind III is a restriction enzyme for rapid DNA digestion, including plasmid, genomic DNA as well as PCR products.

Hind III 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) |
| Hind III                       | 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  |
| Hind III                       | 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 |
|------|-------|--------|-------|----------|------|------------|--------|
| 6    | 0     | 1      | 1     | 1        | 6    | 1          | 12     |

## 5 Methylation Effects on Digestion

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

## 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.