

## EcoR I

Store at  $-20^{\circ}\text{C}$

| Cat. nos. | Size         | Conc.                  | Lot no. | Exp. Date |
|-----------|--------------|------------------------|---------|-----------|
| 15202-013 | 5,000 units  | 8–20 U/ $\mu\text{L}$  |         |           |
| 15202-039 | 20,000 units | 30–60 U/ $\mu\text{L}$ |         |           |

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### Restriction Site

5' -G↓AATTC-3'

3' -CTTAA↑G-5'

Cleavage at the sequence GAATTCG can be blocked by CG methylase.

### Unit Definition

One unit is the amount of enzyme required to completely digest 1  $\mu\text{g}$  of  $\lambda$  DNA in 50  $\mu\text{L}$  of the reaction mixture in 1 hour at  $37^{\circ}\text{C}$ .

### Components

| Item               | 15202-013  | 15202-039       | Storage               |
|--------------------|------------|-----------------|-----------------------|
| EcoR I Enzyme      | 5000 units | 20,000 units    | $-20^{\circ}\text{C}$ |
| 10X Buffer H       | 1 mL       | 2 $\times$ 1 mL | $-20^{\circ}\text{C}$ |
| 10X Loading Buffer | 1 mL       | 1 mL            | Room temp.            |

### Buffers

**Storage Buffer:** 100 mM KCl, 0.1 mM EDTA, 1 mM Dithiothreitol (DTT), 10 mM Tris-HCl, pH 7.5, 0.15% Triton X-100™, 0.01% BSA, 50% (v/v) glycerol

**10X Buffer H:** 0.5 M Tris-HCl, pH 7.5, 1 M NaCl, 100 mM  $\text{MgCl}_2$ , 10 mM DTT

**10X Loading Buffer:** 1% SDS, 50% glycerol, 0.05% Bromophenol blue

**Note:** SDS in the loading buffer may precipitate during storage at room temperature. Warm the buffer to dissolve any SDS precipitate before use.

**For Research Use Only.** Not for use in diagnostic procedures.

## Relative Activity in Universal Buffers

| Buffer                | L   | M    | H   | K    | T (+BSA) |
|-----------------------|-----|------|-----|------|----------|
| Relative Activity (%) | 20* | 100* | 100 | 120* | 80*      |

\*Weak star activity is detected. Unrelated sites may be cut in the presence of high concentrations of glycerol or  $Mn^{2+}$ , and at low ionic strength. Addition of spermine (0.2 mM) reduces star activity 50–70% while only reducing enzyme activity 20–30%.

## Perform Restriction Digest

1. Prepare a reaction mix with the following components in a clean tube:

|               |                  |
|---------------|------------------|
| EcoR I        | 1 $\mu$ L        |
| 10X Buffer H  | 2 $\mu$ L        |
| Substrate DNA | $\leq$ 1 $\mu$ g |
| Sterile water | to 20 $\mu$ L    |

2. Incubate at 37°C (See “Unit Definition” for details).
3. (Optional) Inactivate enzyme by heating at 65°C for 20 minutes.
4. (Optional) Add 1/10 volume of 10X Loading Buffer to an aliquot of the reaction mix, and analyze by agarose gel electrophoresis.

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