



## Product Information

### RESTRICTION ENDONUCLEASE EcoR I

Product No. **R 4640**

Store at 0 to -20 °C

#### Product Summary

Recognition Sequence: 5' G/AATTC'3

Activity: 40,000 units/ml

Cutting: 100%

Ligation: >95%

Recutting: >95%

No degradation detected with >100 units for 16 hrs.

Fold over digestion: 1,600 (100 units x 16 hrs.)

Package Size: 1,000 units and 5,000 units

#### Unit Definition

One unit is the enzyme activity that completely cleaves 1 µg λDNA in 1 hr. at 37 °C in a total volume of 50 µl of Buffer SH for restriction enzymes. 1 µg pBR322 DNA is digested completely by 2 units of Eco RI.

#### Specificity

Eco RI recognizes the sequence G/AATTC and generates fragments with 5'-cohesive termini.<sup>1</sup> Eco RI is an isoschizomer to Rsa I. Eco RI is inhibited by the presence of N<sup>6</sup>-methyladenine at either or both A residues in the sequence G<sup>m</sup>A<sup>m</sup>ATTC.

#### Comments

Digestion Buffer SH is supplied as a 10x concentrate.

0.5-100 units of Eco RI is not heat inactivated after incubation at 65 °C for 15 min.

#### Eco RI Storage and Dilution Buffer Composition

10 mM Tris-HCl

200 mM NaCl

1.0 mM EDTA

0.5 mM dithioerythritol

0.2% Triton<sup>®</sup> X-100

50% (v/v) glycerol

pH 7.0

#### 1x Digestion Buffer SH (B 3657) Composition for Eco RI: 100% Digestion at 37 °C.

50mM Tris-HCl

100 mM NaCl

10 mM MgCl<sub>2</sub>

1 mM dithioerythritol

pH 7.5

#### Quality Control Testing

##### Absence of unspecific endonuclease activities:

1 µg λDNA is incubated for 16 hrs. in 50 ml buffer SH with >100 units of Eco RI.

#### Ligation and Recutting Assay

Eco RI fragments obtained by complete digestion of 1 µg λDNA are adjusted to pH 7.5 at 20 °C. The Eco RI fragments are then ligated with 0.1 unit T4-DNA ligase at pH 7.5 at 4 °C. A 10 µl reaction mixture, incubated for 16 hrs. at 4 °C, contained: 0.1 units T4-DNA ligase, 66 mM Tris-HCl, 5 mM MgCl<sub>2</sub>, 1 mM dithioerythritol, and 1 mM ATP.

The degree of ligation and subsequent recutting with Eco RI to yield the typical pattern of λxEcoRI fragments is determined.

#### References

1. Hedgpeth, J., et al., Proc. Natl. Acad. Sci USA, **69**, 3448 (1972).