

## G Fast Gene®

# Restriction Enzyme EcoRI



Cat.# FG-EcoRI



Conc. 20 units/µl

Store at -20°C

Supplied with: FastGene® 10XBuffer EcoR I (FG-REBEcoRI) FastGene® 10X FastCut Buffer (FG-REBHF) 6X DNA Loading Buffer Sterile water

#### **Recognition site**

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

**ISO**9001

## **Heat Inactivation**

EcoR I can be inactivated at 65°C for 20 min.

#### Methylation sensitivity

*dam* methylation: Not sensitive *dcm* methylation: Not sensitive CpG methylation: Conditionally Sensitive

#### **Prolonged incubation**

A minimum amount of enzyme required to digest 1  $\mu g$  substrate DNA for 16 hr; 0.13 U.

#### Relative activity in FastGene® Buffers

FastGene®	Buffer I:	50%
FastGene®	Buffer II:	100%
FastGene®	Buffer III:	75%
FastGene®	Buffer IV:	100%
FastGene®	FastCut Buffer:	100%

#### Note

It can cleave non-specifically the sequences of AATT or PuPuATPyPy (Pu=purine; Py=pyrimidine) (star activity). The addition of 2 mM  $\beta$ -mercaptoethanol may inhibit this star activity, Long-term storage can result in aggregation, leading to reduced activity. Both DNA and RNA in a DNA/RNA hybrid duplex can be cleaved sequence specifically. Cleavage of mammalian genomic DNA is impaired by overlapping CpG methylation. Reaction condition of low salt, excess enzyme, excess glycerol (>5%) or high pH (>8.0) can result in star activity.

## Source: E. coli RY13

#### **Reaction conditions**

1X FastGene<sup>®</sup> Buffer EcoR I, 37°C 2X FastGene<sup>®</sup> FastCut Buffer, 37°C

### FastGene® FastCut Buffer

FastGene<sup>®</sup> restriction enzyme can cut substrate DNA in 5-15 min with FastGene<sup>®</sup> FastCut Buffer.

#### 1X FastGene® Buffer EcoR I

100 mM Tris-HCl (pH 7.5 at 25°C) 50 mM NaCl 10 mM MgCl<sub>2</sub> 0.025% Triton X-100

#### Unit definition

One unit is defined as the amount of enzyme required for complete digestion of 1  $\mu$ g bacteriophage  $\lambda$  at 37°C for 1 hr in 50  $\mu$ l reaction mixtures.

#### Quality control

- Unit definition assay
- Overdigestion assay
- Endonuclease assay
- Extreme pure assay

#### Dilution buffer

FastGene® Diluent C

### Standard reaction condition

- Normal protocol

Component	Final Conc.	Volume
Substrate DNA	1 µg	X µl
10X FastGene <sup>®</sup> Buffer EcoR I	1 X	5 µl
EcoR I	20 unit	1 µl
Sterile water		up to 50 µl

→ Incubate at 37°C for 1 hr

#### - Fast protocol

Component	Final Conc.	Volume
Substrate DNA	1 µg	Χ μΙ
10X FastGene <sup>®</sup> FastCut Buffer	2 X	10 µl
EcoR I	20 unit	1 µl
Sterile water		up to 50 µl

→ Incubate at 37°C for 15 min

% We recommend 5-10 units of enzyme per  $\mu$ g DNA and 10-20 units for genomic DNA in a 1 h digest.