Fermentas
LIFE SCIENCES

**CERTIFICATE OF ANALYSIS**

# EcoRI

**#ER0273** HC, 25000 u

**Lot:** **Expiry Date:**

5'...**G↓A A T T C**...3'
3'...**C T T A A↑ G**...5'

Concentration: 50 u/µl
Source: *E.coli* that carries the cloned *ecoRIR* gene from *Escherichia coli* RY13
Supplied with: 5x1 ml of 10X Buffer EcoRI
1 ml of 10x Buffer Tango™

**Store at -20°C**


In total 7 vials. BSA included: Lot# BSA62-313P

ISO 9001 ISO 14001
www.fermentas.com

## RECOMMENDATIONS

**1X Buffer EcoRI** (for 100% EcoRI digestion)
50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 100 mM NaCl, 0.02% Triton X-100, 0.1 mg/ml BSA.

**Incubation temperature**
37°C.

**Unit Definition**
One unit is defined as the amount of EcoRI required to digest 1 µg of lambda DNA in 1 hour at 37°C in 50 µl of recommended reaction buffer.

**Dilution**
Dilute with the Dilution Buffer (#B19): 10 mM Tris-HCl (pH 7.4 at 25°C), 100 mM KCl, 1 mM EDTA, 1 mM DTT, 0.2 mg/ml BSA and 50% glycerol.

**Double Digests**
Tango™ Buffer is provided to simplify buffer selection for double digests. 98% of Fermentas restriction enzymes are active in a 1X or 2X concentration of Tango™ Buffer. Please refer to the Fermentas Catalog or go to www.fermentas.com/doubledigest to choose the best buffer for your experiments.
1X Tango™ Buffer:
33 mM Tris-acetate (pH 7.9 at 37°C), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/ml BSA.

## Storage Buffer

EcoRI is supplied in: 10 mM potassium phosphate (pH 7.4 at 25°C), 300 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.2 mg/ml BSA, 0.15% Triton X-100 and 50% glycerol.

## Recommended Protocol for Digestion

- Add:

| | |
|---|---|
| nuclease-free water | 16 µl |
| 10X Buffer EcoRI | 2 µl |
| DNA (0.5-1 µg/µl) | 1 µl |
| EcoRI | 0.5-2 µl[a, b] |

- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours[b].

The digestion reaction may be scaled either up or down.

## Recommended Protocol for Digestion of PCR Products Directly after Amplification

- Add:

| | |
|---|---|
| PCR reaction mixture | 10 µl (~0.1-0.5 µg of DNA) |
| nuclease-free water | 18 µl |
| 10X Buffer EcoRI | 2 µl |
| EcoRI | 1-2 µl[a, b] |

- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours[b].

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[a] This volume of the enzyme is recommended for preparations of standard concentrations (10 u/µl), whereas HC enzymes (50 u/µl) should be diluted with the Dilution Buffer to obtain 10 u/µl concentration.

[b] *See* Note.

## Thermal Inactivation

EcoRI is inactivated by incubation at 65°C for 20 min.

# ENZYME PROPERTIES

## Enzyme Activity in Fermentas REase Buffers, %

| EcoRI | B | G | O | R | Tango™ | 2X Tango™ |
|---|---|---|---|---|---|---|
| **100** | 0-20 | NR | 100 | 100* | NR | 100 |

*Star activity appears at a greater than 5-fold overdigestion (5 u x 1h).
NR: buffer is not recommended, because of high star activity.

## Methylation Effects on Digestion

Dam: never overlaps – no effect.
Dcm: never overlaps – no effect.
CpG: may overlap – cleavage impaired.
EcoKI: never overlaps – no effect.
EcoBI: may overlap – no effect.

## Stability during Prolonged Incubation

A minimum of 0.2 units of the enzyme is required for complete digestion of 1 µg of lambda DNA in 16 hours at 37°C.

## Digestion of Agarose-embedded DNA

A minimum of 5 units of the enzyme is required for complete digestion of 1 µg of agarose-embedded lambda DNA in 16 hours.

## Compatible Ends

XapI, MunI, TasI

## Number of Recognition Sites in DNA

| λ | ΦX174 | pBR322 | pUC57 | pUC18/19 | pTZ19R/U | M13mp18/19 |
|---|---|---|---|---|---|---|
| 5 | 0 | 1 | 1 | 1 | 1 | 1 |

## Note

A large excess of EcoRI (7.5 u/µg DNA x 16 hours), low salt concentration, high pH, or the replacement of $Mg^{2+}$ by $Mn^{2+}$ may result in star activity.

For **QUALITY CONTROL ASSAY DATA** *see* back page

# QUALITY CONTROL ASSAY DATA

## Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 80-fold overdigestion with EcoRI (5 u/µg lambda DNA x 16 hours).

## Ligation/Recutting Assay

After a 50-fold overdigestion (3 u/µg DNA x 17 hours) with EcoRI, more than 95% of the digested DNA fragments can be ligated at a 5'-termini concentration of 0.02 µM. More than 95% of these sites can be recut.

## Labeled Oligonucleotide (LO) Assay

No detectable degradation of single-stranded or double-stranded labeled oligonucleotides occurred during incubation with 10 units of EcoRI for 4 hours.

## Blue/White Cloning Assay

pUC57 was incubated with 10 units of EcoRI for 16 hours. After religation and transformation, the background level of white colonies was 0.1%.

**Quality authorized by:**  Jurgita Zilinskiene

**PRODUCT USE LIMITATION.**

This product is developed, designed and sold exclusively *for research purposes and in vitro use only.* The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
Please refer to www.fermentas.com for Material Safety Data Sheet of the product.

(5) Revised 29.08.2006