

PRODUCT INFORMATION

EcoRI

#ER0271 5000 u

Lot: **Expiry Date:**

5'...G↓A A T T C...3'

3'...C T T A A↑G...5'

Concentration: 10 u/μl

Source: *E.coli* that carries the cloned *ecoRIR* gene from *Escherichia coli* RY13

Supplied with: 2 x 1 ml of 10X Buffer EcoRI
1 ml of 10x Buffer Tango

Store at -20°C



In total 4 vials.

BSA included

www.thermoscientific.com/fermentas

RECOMMENDATIONS

1X Buffer EcoRI (for 100% EcoRI digestion)

50 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 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

Thermo Scientific Tango Buffer is provided to simplify buffer selection for double digests. 98% of Thermo Scientific restriction enzymes are active in a 1X or 2X concentration of Tango™ Buffer. Please refer 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*, **
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours**.

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*, **
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours**.

* 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.

** See Overdigestion Assay.

Thermal Inactivation

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

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific 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

For **CERTIFICATE OF ANALYSIS** see back page

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after an 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 0.05 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 <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.thermoscientific.com/fermentas for Material Safety Data Sheet of the product.

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