Thermo SCIENTIFIC

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: 2x1 mL of 10X Buffer EcoRI
1 mL of 10x Buffer Tango

Store at -20°C

In total 4 vials. BSA included

www.thermoscientific.com/onebio

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
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.thermoscientific.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.

Rev.9.

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 µ***,****

- 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 × 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 × 16 hours).

Ligation and Recleavage (L/R) Assay

The ligation and recleavage assay was replaced with LO test after validating experiments showed LO test ability to trace nuclease and phosphatase activities with sensitivity that is higher than L/R by a factor of 100.

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 (B/W) Cloning Assay

The B/W assay was replaced with LO test after validating experiments showed LO test ability to detect nuclease and phosphatase activities with sensitivity that equals to that of B/W test.

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/onebio for Material Safety Data Sheet of the product.