Thermo SCIENTIFIC

PRODUCT INFORMATION

EcoRII

#ER1921 200 U

Lot: ___ **Expiry Date: _**

5'…↓**C C W G G** …3'
3'… **G G W C C**↑…5'

Concentration:	10 U/µL
Source:	*E.coli* that carries the cloned *ecoRIIR* gene from *E.coli* R245
Supplied with:	1 mL of 10X Buffer O 1 mL of 10X Buffer Tango

Store at -20°C

BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Buffer O (for 100% EcoRII digestion)
50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 100 mM NaCl, 0.1 mg/mL BSA.

Incubation temperature
37°C.

Unit Definition
One unit is defined as the amount of EcoRII required to digest 1 µg of pBR322 DNA *dcm*⁻ in 1 hour at 37°C in 50 µL of recommended reaction buffer.

Dilution
Dilute with 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.

Storage Buffer

EcoRII is supplied in: 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.

Recommended Protocol for Digestion

- Add:

nuclease-free water	16 µL
10X Buffer O	2 µL
DNA (0.5-1 µg/µL)	1 µL
EcoRII	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 O	2 µL
EcoRII	1-2 µL*

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

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* *See* Overdigestion Assay.

Thermal Inactivation

EcoRII is inactivated by incubation at 80°C for 20 min.

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

B	G	O	R	Tango	2X Tango
20-50	50-100	**100**	50-100	20-50	50-100

Methylation Effects on Digestion

Dam: never overlaps – no effect.
Dcm: completely overlaps – blocked.
CpG: never overlaps – no effect.
EcoKI: never overlaps – no effect.
EcoBI: never overlaps – no effect.

Stability during Prolonged Incubation

A minimum of 0.1 units of the enzyme is required for complete digestion of 1 µg of lambda DNA in 16 hours at 37°C.

Compatible Ends

BssKI (↓CCWGG), PfoI (T↓CCWGGA), SexAI.

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
70	2	6	5	5	5	7

For **CERTIFICATE OF ANALYSIS** *see* back page

Note

- At least two copies of EcoRII recognition site are required for efficient cleavage. For cleavage of DNA substrates with only one copy of recognition site MvaI (#ER0551), neoschizomer of EcoRII, is recommended.
- EcoRII may remain associated with the cleaved DNA. This may cause DNA band shifting during electrophoresis. To avoid atypical DNA band patterns, use the 6X DNA Loading Dye&SDS Solution (#R1151) for sample preparation or heat the digested DNA in the presence of SDS prior to electrophoresis.
- EcoRII is blocked by overlapping *dcm* methylation. To avoid *dcm* methylation, use a *dam*$^-$, *dcm*$^-$ strain such as GM2163 (#M0099).

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 10-fold overdigestion with EcoRII (10 U/µg pBR322 DNA *dcm*$^-$ x 1 hour).

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 EcoRII for 4 hours.

Quality authorized by:  Jurgita Zilinskiene