Thermo scientific

PRODUCT INFORMATION Eco47I (AvaII) #ER0311 800 U

Lot: ____ Expiry Date: _

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

Concentration: Source:

10 U/µL *E.coli* that carries the cloned *eco47IR* gene from *E.coli* RFL47 1 mL of 10X Buffer R 1 mL of 10X Buffer Tango

Supplied with:

Store at -20°C **R** 37° **D**cm **CG** $20^{\circ}_{65^{\circ}}$ **D LO**

In total 3 vials.

BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Buffer R (for 100% Eco47I digestion)
10 mM Tris-HCI (pH 8.5), 10 mM MgCl₂, 100 mM KCI,
0.1 mg/mL BSA.

Incubation temperature

37°C.

Unit Definition

One unit is defined as the amount of Eco47I required to digest 1 μg of lambda DNA 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

<u>www.thermoscientific.com/doubledigest</u> 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

Eco47I is supplied in: 10 mM Tris-HCI (pH 7.4 at 25°C), 100 mM KCI, 1 mM DTT, 1 mM EDTA, 0.2 mg/mL BSA and 50% glycerol.

Recommended Protocol for Digestion

• Add:

nuclease-free water	16 µL
10X Buffer R	2 µL
DNA (0.5-1 µg/µL)	1 µL
Eco47I	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	$10~\mu L~$ (~0.1-0.5 μg of DNA)
nuclease-free water	18 μL
10X Buffer R	2 µL
Eco47I	1-2 μL

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

Thermal Inactivation

Eco47I is inactivated by incubation at 65° C for 20 min.

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

В	G	0	R	Tango	2X Tango
0-20	50-100	50-100	100	50-100	50-100

Methylation Effects on Digestion

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

Stability during Prolonged Incubation

A minimum of 0.3 units of the enzyme is required for complete digestion of 1 μ g of lambda DNA in 16 hours at 37°C.

Compatible Ends

Cfr13I, Cpol, Psp5II, SanDI

Number of Recognition Sites in DNA

λ	ФХ174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
35	1	8	2	2	2	1

Note

Eco47I 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 160-fold overdigestion with Eco47I (10 U/µg lambda DNA \times 16 hours).

Ligation and Recleavage (L/R) Assay

The ligation and recleavage assay was replaced with L0 test after validating experiments showed L0 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 doublestranded labeled oligonucleotides occurred during incubation with 10 units of Eco47I for 4 hours.

Quality authorized by:



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 <u>www.thermoscientific.com/onebio</u> for Material Safety Data Sheet of the product.

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