

CERTIFICATE OF ANALYSIS

# Eco57I (AcuI)

#ER0342 1000 u

Lot: Expiry Date:

5'... C T G A A G (N)<sub>16</sub> ↓...3'  
3'... G A C T T C (N)<sub>14</sub> ↑...5'

Concentration: 5 u/μl  
Source: *E.coli* that carries the cloned *eco57IR* gene from *E.coli* RFL57  
Supplied with: 1 ml of 10X Buffer G  
1 ml of 10X Buffer Tango™  
2x0.1 ml of 50X SAM (0.5 mM)

Store at -20°C



In total 5 vials.

BSA included: Lot# BSA62-313P

## RECOMMENDATIONS

[1X Buffer G]+SAM\* (for 100% Eco57I digestion)

[10 mM Tris-HCl (pH 7.5), 10 mM MgCl<sub>2</sub>, 50 mM NaCl, 0.1 mg/ml BSA] + 0.01 mM S-adenosylmethionine (SAM).

### Incubation temperature

37°C.

### Unit Definition

One unit is defined as the amount of Eco57I at which no change in the fragmentation pattern is observed with further increase of enzyme. 1 μg of lambda DNA is incubated with Eco57I for 1 hour at 37°C in 50 μl of recommended reaction buffer.

### Dilution

Dilute with Dilution Buffer (#B19): 10mM 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](http://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.

\* Eco57I requires only Mg<sup>2+</sup> for its activity, but is stimulated by S-adenosylmethionine. 10 μM S-adenosylmethionine gives more than a 100-fold increase in Eco57I activity. Still, a complete cleavage of some substrates with Eco57I is difficult to achieve.

## Storage Buffer

Eco57I is supplied in: 10 mM potassium phosphate (pH 7.4 at 25°C), 100 mM NaCl, 7 mM 2-mercaptoethanol, 1 mM EDTA and 50% glycerol.

## Recommended Protocol for Digestion

- Add:

nuclease-free water	16 µl
10X Buffer G	2 µl
DNA (0.5-1 µg/µl)	1 µl
50X SAM	0.4 µl
Eco57I	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 G	2 µl
50X SAM	0.6 µl
Eco57I	1-2 µl*
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours\*.

\* See Note.

## Thermal Inactivation

Eco57I is inactivated by incubation at 65°C for 20 min.

## ENZYME PROPERTIES

### Enzyme Activity in Fermentas REase Buffers, %

B <sub>+SAM</sub>	G <sub>+SAM</sub>	O <sub>+SAM</sub>	R <sub>+SAM</sub>	Tango™ <sub>+SAM</sub>	2X Tango™ <sub>+SAM</sub>
100	100	20-50	20-50	50-100	50-100

### Methylation Effects on Digestion

Dam: never overlaps – no effect.  
Dcm: never overlaps – no effect.  
CpG: never overlaps – no effect.  
EcoKI: never overlaps – no effect.  
EcoBI: may overlap – effect not determined.

### Stability during Prolonged Incubation

A minimum of 1.0 unit of the enzyme is required for digestion of 1 µg of lambda DNA in 16 hours at 37°C.

### Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
40	0	2	2	2	2	0

### Note

A large excess of Eco57I (7.5 u/µg DNA x 16 hours) 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 an 80-fold overdigestion with Eco57I (5 u/μg lambda DNA x 16 hours).

## Ligation/Recutting Assay

After a 16-fold overdigestion (1 u/μg DNA x 16 hours) with Eco57I, approximately 70% of the digested DNA fragments can be ligated at a 5'-termini concentration of 0.2 μM. None of these can be recut due to the methylation of the recognition sequence by Eco57I.

## Labeled Oligonucleotide (LO) Assay

No detectable degradation of single-stranded or double-stranded labeled oligonucleotides occurred during incubation with 10 units of Eco57I for 4 hours.

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](http://www.fermentas.com) for Material Safety Data Sheet of the product.