

CERTIFICATE OF ANALYSIS

Eco57MI

#ER1671 50 u

Lot: Expiry Date:

5'... C T G P u A G (N)₁₆ ↓...3'

3'... G A C P y T C (N)₁₄ ↑...5'

Concentration: 2 u/μl

Source: *E. coli* that carries the cloned *eco57IR* mutant gene from *Escherichia coli* RFL57, encoding protein with altered specificity

Supplied with: 1 ml of 10X Buffer B
1 ml of 10X Buffer Tango™
0.1 ml of 50X SAM (0.05 mM)

Store at -20°C



In total 4 vials.

BSA included: Lot# BSA62-313P

RECOMMENDATIONS

[1X Buffer B]+SAM* (for 100% Eco57MI digestion)
[10 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 0.1 mg/ml BSA] +
1 μM S-adenosylmethionine (SAM).

Incubation temperature

37°C.

Unit Definition

One unit is defined as the amount of Eco57MI at which no change in the fragmentation pattern is observed with further increase of enzyme. 1 μg of lambda DNA is incubated with Eco57MI for 1 hour at 37°C in 50 μl of recommended reaction buffer.

Dilution

Dilute with Dilution Buffer (#B19): 10 mM Tris-HCl (pH7.4 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 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.

* Eco57MI requires only Mg²⁺ for its activity, but is stimulated by S-adenosylmethionine. 1 μM S-adenosylmethionine gives more than a 100-fold increase in Eco57MI activity. Still, a complete cleavage of some substrates with Eco57MI is difficult to achieve.

Storage Buffer

Eco57MI is supplied in: 10 mM potassium phosphate (pH7.4 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 B	2 µl
DNA (0.5-1 µg/µl)	1 µl
50X SAM	0.4 µl
Eco57MI	0.5-2 µl*
- Mix gently and spin down for a few seconds.
- Incubate at 55°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 B	2 µl
50X SAM	0.6 µl
Eco57MI	1-2 µl*
- Mix gently and spin down for a few seconds.
- Incubate at 55°C for 1-16 hours*.

* See Note.

Thermal Inactivation

Eco57MI is inactivated by incubation at 65°C for 20 min.

ENZYME PROPERTIES

Enzyme Activity in Fermentas REase Buffers, %

<u>B_{+SAM}</u>	<u>G_{+SAM}</u>	<u>O_{+SAM}</u>	<u>R_{+SAM}</u>	<u>Tango™_{+SAM}</u>	<u>2X Tango™_{+SAM}</u>
100	50-100	0-20	20-50	50-100	0-20

Methylation Effects on Digestion

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

<u>λ</u>	<u>ΦX174</u>	<u>pBR322</u>	<u>pUC57</u>	<u>pUC18/19</u>	<u>pTZ19R/U</u>	<u>M13mp18/19</u>
65	3	6	3	3	3	2

Note

A large excess of Eco57MI (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 a 80-fold overdigestion with Eco57MI (5 u/μg lambda DNA x 16 hours).

Ligation/Recutting Assay

After a 16-fold overdigestion (1 u/μg DNA x 16 hours) with Eco57MI, approximately 90% of the digested DNA fragments can be ligated at a 5'-termini concentration of 0.6 μM. None of these can be recut due to the methylation of the recognition sequence by Eco57MI.

Labeled Oligonucleotide (LO) Assay

No detectable degradation of single-stranded or double-stranded labeled oligonucleotides occurred during incubation with 10 units of Eco57MI for 4 hours.

Quality authorized by:



Jurgita Zilinskiene

Note

This product and process is covered by US patent No 6893854 and corresponding counterparts.

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 for Material Safety Data Sheet of the product.