Fermentas
LIFE SCIENCES

CERTIFICATE OF ANALYSIS

Eco47I (AvaII)

#ER0312 4000 u

Lot: **Expiry Date:**

```
          T
5'...G↓G  A  C  C...3'
3'...C  C  T  G↑G...5'
          A
```

Concentration: 10 u/µl
Source: *E.coli* that carries the cloned *eco47IR* gene from *E.coli* RFL47
Supplied with: 2x1 ml of 10X Buffer R
1 ml of 10X Buffer Tango™

Store at -20°C

In total 4 vials. BSA included: Lot# BSA62-313P

ISO 9001 ISO 14001
www.fermentas.com

RECOMMENDATIONS

1X Buffer R (for 100% Eco47I digestion)
10 mM Tris-HCl (pH 8.5), 10 mM $MgCl_2$, 100 mM KCl, 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
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.

Storage Buffer

Eco47I is supplied in: 10 mM Tris-HCl (pH 7.4 at 25°C), 100 mM KCl, 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 µl	(~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 Fermentas REase Buffers, %

B	G	O	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 µg of lambda DNA in 16 hours at 37°C.

Compatible Ends

Cfr13I, CpoI, Psp5II, SanDI

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
35	1	8	2	2	2	1

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 160-fold overdigestion with Eco47I (10 u/µg lambda DNA x 16 hours).

Ligation/Recutting Assay

After a 50-fold overdigestion (3 u/µg DNA x 17 hours) with Eco47I, more than 95% of the digested DNA fragments can be ligated at a 5'-termini concentration of 0.5 µM. More than 95% of these sites can be recut.

Labeled Oligonucleotide (LO) Assay

No detectable degradation of single-stranded or double-stranded labeled oligonucleotides occurred during incubation with 10 units of Eco47I for 4 hours.

Quality authorized by:  Jurgita Zilinskiene

(5) Revised 28.08.2006