

CERTIFICATE OF ANALYSIS

Eco72I (PmlI)

#ER0361 2000 u

Lot: Expiry Date:

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

Concentration: 10 u/μl
Source: *E.coli* that carries the cloned
eco72IR gene from *E.coli* RFL72
Supplied with: 1 ml of 10X Buffer Tango™

Store at -20°C



In total 2 vials.

BSA included: Lot# BSA62-313P

RECOMMENDATIONS

1X Buffer Tango™ (for 100% Eco72I digestion)
33 mM Tris-acetate (pH 7.9), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/ml BSA.

Incubation temperature
37°C.

Unit Definition

One unit is defined as the amount of Eco72I 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.

Storage Buffer

Eco72I 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 μ l
10X Buffer Tango™	2 μ l
DNA (0.5-1 μ g/ μ l)	1 μ l
Eco72I	0.5-2 μ l*
- 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 μ l (~0.1-0.5 μ g of DNA)
nuclease-free water	18 μ l
10X Buffer Tango™	2 μ l
Eco72I	1-2 μ l*
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours*.

* See Star Activity.

Thermal Inactivation

Eco72I 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™
NR	NR	0-20	0-20	100	20-50

NR – buffer is not recommended, because of high star activity.

Star Activity

An excess of Eco72I (15 u/ μ g DNA x 1 hour) or low salt concentration may result in star activity.

Methylation Effects on Digestion

Dam: never overlaps – no effect.

Dcm: never overlaps – no effect.

CpG: completely overlaps – blocked.

EcoKI: may overlap – effect not determined.

EcoBI: may overlap – effect not determined.

Stability during Prolonged Incubation

A minimum of 0.5 units of the enzyme is required for complete digestion of 1 μ g of lambda DNA in 16 hours at 37°C.

Digestion of Agarose-embedded DNA

A minimum of 5 units of the enzyme is required for complete digestion of 1 μ g of agarose-embedded lambda DNA in 16 hours.

Number of Recognition Sites in DNA

λ	Φ X174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
3	0	0	0	0	0	0

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 10-fold overdigestion with Eco72I (10 u/μg lambda DNA x 1 hour) (see Star Activity).

Ligation/Recutting Assay

After a 10-fold overdigestion (5u/μg DNA x 2 hours) with Eco72I, more than 80% of the digested DNA fragments can be ligated in a reaction mixture containing 20-40 u of T4 DNA ligase/1 μg of fragments and 10% PEG at a 5'-termini concentration of 0.03 μM. More than 90% 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 Eco72I for 4 hours.

Blue/White Cloning Assay

A mixture of pUC57/HindIII, pUC57/Eco32I and pUC57/PstI digests was incubated with 10 units of Eco72I for 16 hours. After religation and transformation, the background level of white colonies was 0.1%.

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 for Material Safety Data Sheet of the product.