

CERTIFICATE OF ANALYSIS

Eco31I (BsaI)

#ER0291 1000 u

Lot: Expiry Date:

5'...**G G T C T C (N)**₁↓...3'
3'...**C C A G A G (N)**₅↑...5'

Concentration: 10 u/μl
Source: *E.coli* that carries the cloned *eco31I*R gene from *E.coli* RFL31
Supplied with: 1 ml of 10X Buffer G
1 ml of 10X Buffer Tango™

Store at -20°C



In total 3 vials.

BSA included: Lot# BSA62-313P

RECOMMENDATIONS

1X Buffer G (for 100% Eco31I digestion)

10 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 50 mM NaCl, 0.1 mg/ml BSA.

Incubation temperature

37°C.

Unit Definition

One unit is defined as the amount of Eco31I required to digest 1 μg of lambda DNA *dcm*⁻-HindIII fragments in 1 hour at 37°C in 50 μl of recommended reaction buffer. Assayed using lambda DNA *dcm*⁻, as one of two Eco31I recognition sites is difficult to cleave.

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

Eco31I is supplied in: 10 mM Tris-HCl (pH 7.5 at 25°C), 200 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.2 mg/ml BSA 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
Eco31I	0.5-2 µl*
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours*.

Recommended Protocol for Digestion of PCR Products Directly after Amplification

- Add:

PCR reaction mixture	10 µl (about 1 µg of DNA)
Water, nuclease-free	16 µl
10X Buffer G	2 µl
Eco31I	1-2 µl*
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours*.

* See Note.

Thermal Inactivation

Eco31I 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™
50-100	100	0-20	0-20	50-100	20-50

Methylation Effects on Digestion

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

Stability during Prolonged Incubation

A minimum of 0.3 units of the enzyme is required for complete digestion of 1 µg of lambda DNA *dcm*⁻ 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 µg of agarose-embedded lambda DNA *dcm*⁻ in 16 hours.

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
2	0	1	1	1	1	0

Note

A large excess of Eco31I (10 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 Eco31I (5 u/μg lambda DNA x 16 hours).

Ligation/Recutting Assay

After a 50-fold overdigestion (3 u/μg DNA x 17 hours) with Eco31I, more than 95% of the digested DNA fragments can be ligated at a 5'-termini concentration of 0.05 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 Eco31I for 4 hours.

Quality authorized by:



Jurgita Zilinskiene

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