

CERTIFICATE OF ANALYSIS

EheI (NarI*)

#ER0441 500 u

Lot: **Expiry Date:**

5'...G G C↓G C C...3'

3'...C C G↑C G G...5'

* EheI - neoschizomer of NarI, completely digests lambda and pBR322 DNAs and produces fragments that have blunt ends.

Concentration: 10 u/μl
Source: *Erwinia herbicola* 9/5
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% EheI 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 EheI required to digest 1 μg of lambda DNA-PstI fragments 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

EheI is supplied in: 10 mM Tris-HCl (pH 7.5 at 25°C), 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 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
Ehel	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
Ehel	1-2 μ l**
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours**.

** See Note.

Thermal Inactivation

Ehel 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™
20-50	50-100	0-20	0-20	100	20-50

Methylation Effects on Digestion

Dam: never overlaps – no effect.
Dcm: may overlap – no effect.
CpG: completely overlaps – blocked.
EcoKI: never overlaps – no effect.
EcoBI: never overlaps – no effect.

Stability during Prolonged Incubation

A minimum of 1.0 unit 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 20 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
1	2	4	1	1	0	1

Note

A large excess of Ehel (10 u/ μ g DNA x 16 hours) may result in star activity.

QUALITY CONTROL ASSAY DATA

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 80-fold overdigestion with Ehel (5 u/μg lambda DNA x 16 hours).

Ligation/Recutting Assay

After a 50-fold overdigestion (3 u/μg DNA x 17 hours) with Ehel, more than 80% of the digested pBR322 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 0.35 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 Ehel for 4 hours.

Quality authorized by:

 Laima Samaliene

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.