

CERTIFICATE OF ANALYSIS

BoxI (PshAI)

#ER1431 500 u

Lot: Expiry Date:

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

Concentration: 10 u/μl
Source: *Brevibacterium oxydans* lti 12-052
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% BoxI 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 BoxI 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.

* Incubation at 30°C results in a 2.5-fold increase in activity.

Storage Buffer

BoxI is supplied in: 10 mM potassium phosphate (pH 7.4 at 25°C), 100 mM NaCl, 1 mM EDTA, 7 mM 2-mercaptoethanol, 0.15% Triton X-100, 0.2 mg/ml BSA, and 50% glycerol.

Recommended Protocol for Digestion

- Add:

| | |
|---------------------|----------|
| nuclease-free water | 16 µl |
| 10X Buffer Tango™ | 2 µl |
| DNA (0.5-1 µg/µl) | 1 µl |
| BoxI | 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 Tango™ | 2 µl |
| BoxI | 1-2 µl |
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours.

Thermal Inactivation

BoxI is inactivated by incubation at 80°C for 20 min.

ENZYME PROPERTIES

Enzyme Activity in Fermentas REase Buffers, %

| B | G | O | R | Tango™ | 2X Tango™ |
|------|------|------|-------|--------|-----------|
| 0-20 | 0-20 | 0-20 | 20-50 | 100 | 20-50 |

Methylation Effects on Digestion

Dam: never overlaps – no effect.
Dcm: never overlaps – no effect.
CpG: may overlap – cleavage impaired.
EcoKI: never overlaps – no effect.
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 µg of lambda DNA in 16 hours at 37°C.

Digestion of Agarose-embedded DNA

A minimum of 10 units of the enzyme is required for complete digestion of 1 µg of agarose-embedded lambda DNA in 16 hours.

Number of Recognition Sites in DNA

| λ | ΦX174 | pBR322 | pUC57 | pUC18/19 | pTZ19R/U | M13mp18/19 |
|---|-------|--------|-------|----------|----------|------------|
| 7 | 1 | 1 | 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 160-fold overdigestion with BoxI (10 u/μg lambda DNA x 16 hours).

Ligation/Recutting Assay

After a 50-fold overdigestion (3 u/μg DNA x 17 hours) with BoxI, more than 90% of the digested DNA fragments can be ligated at a 5'-termini concentration of 0.08 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 BoxI for 4 hours.

Blue/White Cloning Assay

A mixture of pUC57/HindIII, pUC57/PstI and pUC57/Eco32I digests was incubated with 10 units of BoxI 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.