Thermo SCIENTIFIC

PRODUCT INFORMATION

PstI

#ER0611 3000 U

Lot: ___ **Expiry Date: _**

5'...**C T G C A↓G**...3'

3'...**G↑A C G T C**...5'

Concentration: 10 U/µL

Source: *Providencia stuarti*

Supplied with: 2x1 mL of 10X Buffer O
1 mL of 10X Buffer Tango

Store at -20°C

In total 4 vials. BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Buffer O (for 100% PstI digestion)
50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 100 mM NaCl, 0.1 mg/mL BSA.

Incubation temperature
37°C.

Unit Definition
One unit is defined as the amount of PstI required to digest 1 µg of lambda DNA in 1 hour at 37°C in 50 µL of 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
Thermo Scientific Tango Buffer is provided to simplify buffer selection for double digests. 98% of Thermo Scientific restriction enzymes are active in a 1X or 2X concentration of Tango™ Buffer. Please refer to www.thermoscientific.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.

Rev.9

Storage Buffer

PstI is supplied in: 10 mM Tris-HCl (pH 7.4 at 25°C), 200 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 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 O	2 µL
DNA (0.5-1 µg/µL)	1 µL
PstI	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 O	2 µL
PstI	1-2 µL

- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours.

Thermal Inactivation

PstI is not inactivated by incubation at 80°C for 20 min.

Inactivation Procedure

- To prepare the digested DNA for electrophoresis:
  – stop the digestion reaction by adding 0.5 M EDTA, pH 8.0 (#R1021), to achieve a 20 mM final concentration. Mix thoroughly, add an electrophoresis loading dye and load onto gel.
- To prepare DNA suitable for further enzymatic reactions:
  – extract with phenol/chloroform, precipitate with ethanol or isopropanol, wash the pellet with 75% cold ethanol and air-dry;
  – dissolve DNA in either nuclease-free water, TE buffer, or a buffer suitable for further applications;
  – check the DNA concentration in the solution.

For **ENZYME PROPERTIES** and **CERTIFICATE OF ANALYSIS**
see back page

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

B	G	O	R	Tango	2X Tango
50-100	50-100	**100**	100	50-100	50-100

Methylation Effect on Digestion

Dam: never overlaps – no effect.
Dcm: never overlaps – no effect.
CpG: never overlaps – no effect.
EcoKI: never overlaps – no effect.
EcoBI: never overlaps – no effect.

Stability during Prolonged Incubation

A minimum of 0.2 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 5 units of the enzyme is required for complete digestion of 1 µg of agarose-embedded lambda DNA in 16 hours.

Compatible Ends

Alw21I, BseSI, Mph1103I, SdaI, SduI

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
28	1	1	1	1	1	1

Note

- Conditions of high pH, low salt, high glycerol, 8 % DMSO can cause star activity (Malyguine, E., et al., Gene, 8, 163-177, 1980).
- Surrounding sequences: the presence of adjacent runs of G-C base pairs confers significant resistance to cleavage (Armstrong, K. and Bauer, W.R., NAR, 10, 993-1007, 1982).
- 100 % dUTP incorporation at the recognition site reduces PstI cleavage to 25 % (Glenn, T.C., et al., Biotechniques, 17, 1086-1090, 1994).
- PstI will not cut AGCTGCAG when methylated by AluI methyltransferase.

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion with PstI (10 U/µg lambda DNA × 16 hours).

Ligation and Recleavage (L/R) Assay

The ligation and recleavage assay was replaced with LO test after validating experiments showed LO test ability to trace nuclease and phosphatase activities with sensitivity that is higher than L/R by a factor of 100.

Labeled Oligonucleotide (LO) Assay

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

Blue/White (B/W) Cloning Assay

The B/W assay was replaced with LO test after validating experiments showed LO test ability to detect nuclease and phosphatase activities with sensitivity that equals to that of B/W test.

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.thermoscientific.com/onebio for Material Safety Data Sheet of the product.

© 2012 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.