Thermo SCIENTIFIC

PRODUCT INFORMATION

PasI

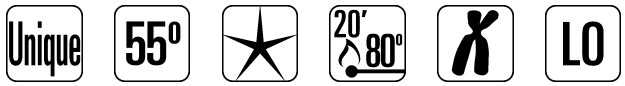
#ER1861 200 U

Lot: __ **Expiry Date: ____**

5'...**C C↓C W G G G**...3'
3'...**G G G W C↑C C**...5'

Concentration: 10 U/µL
Source: *Pseudomonas anquilliseptica* RFL1
Supplied with: 1 mL of 10X Buffer PasI

Store at -20°C



BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Buffer PasI (for 100% PasI digestion)
10 mM Bis-Tris Propane-HCl (pH 6.5), 10 mM $MgCl_2$, 100 mM KCl, 0.1 mg/mL BSA.

Incubation temperature
55°C*.

Unit Definition
One unit is defined as the amount of PasI required to digest 1 µg of lambda DNA-XagI fragments in 1 hour at 55°C in 50 µL of recommended reaction buffer.

Dilution
Dilute with Dilution Buffer: 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
Please refer to the to www.thermoscientific.com/doubledigest to choose the best buffer for your experiments.

Storage Buffer
PasI is supplied in: 10 mM Tris-HCl (pH 7.4 at 25°C), 100 mM KCl, 1 mM DTT, 1 mM EDTA, 0.2 mg/mL BSA and 50% glycerol.

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* Incubation at 37°C results in 30% activity.

Recommended Protocol for Digestion

- Add:

nuclease-free water	16 µL
10X Buffer PasI	2 µL
DNA (0.5-1 µg/µL)	1 µL
PasI	0.5-2 µL**

- Mix gently and spin down for a few seconds.
- Incubate at 55°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 PasI	2 µL
PasI	1-2 µL**

- Mix gently and spin down for a few seconds.
- Incubate at 55°C for 1-16 hours**.

** *See* Star Activity.

Thermal Inactivation

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

ENZYME PROPERTIES

Enzyme Activity in Fermentas REase Buffers

Buffer PasI – 100%. None of the standard buffers is recommended, because of high star activity

Star Activity

A large excess of PasI (15 U/µg DNA x 1 hour) may result in star activity.

Methylation Effects on Digestion

Dam: never overlaps – no effect.
Dcm: completely 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 55°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.

Compatible Ends

TseI

Number of Recognition Sites in DNA

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

For **CERTIFICATE OF ANALYSIS** *see* back page

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 10-fold overdigestion with PasI (10 U/µg lambda DNA x 1 hour) (*see* Star Activity).

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 PasI 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.