Thermo SCIENTIFIC

PRODUCT INFORMATION

PagI (BspHI)

#ER1281 400 U

Lot: _____ Expiry Date: _____

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

Concentration: 10 U/µL
Source: *E.coli* that carries the cloned *pagIR* gene from *Pseudomonas alcaligenes* Sau 14-027
Supplied with: 1 mL of 10X Buffer O

Store at -20°C

BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Buffer O (for 100% PagI 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 PagI 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
Please go to www.thermoscientific.com/doubledigest to choose the best buffer for your experiments.

Storage Buffer
PagI is supplied in: 10 mM Tris-HCl (pH 7.4 at 25°C), 100 mM NaCl, 1 mM DTT, 1 mM EDTA, 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
PagI	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
PagI	1-2 µL

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

Thermal Inactivation

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

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

B	G	O	R	Tango	2X Tango
0-20	50-100	**100**	NR	NR	NR

NR – buffer is not recommended, because of high star activity

Methylation Effects on Digestion

Dam: may overlap – cleavage impaired.
Dcm: never overlaps – no effect.
CpG: never overlaps – no effect.
EcoKI: never overlaps – no effect.
EcoBI: may overlap – cleavage impaired.

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

AflIII, BtgI, Eco130I, FatI, NcoI, PscI

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
8	3	4	3	3	2	1

Note

PagI cleavage is impaired by overlapping *dam* methylation. To avoid *dam* methylation, use a *dam*$^-$, *dcm*$^-$ strain such as GM2163 (#M0099).

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

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion with PagI (10 U/µg lambda DNA x 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 PagI for 4 hours.

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.