



CERTIFICATE OF ANALYSIS

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 *pagI*
gene from *Pseudomonas alcaligenes*
Sau 14-027

Supplied with: 1 ml of 10X Buffer O

Store at -20°C



In total 2 vials.

BSA included: Lot# BSA62-313P

RECOMMENDATIONS

1X Buffer O (for 100% PagI digestion)

50 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 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 refer to the Fermentas Catalog or go to www.fermentas.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 μ l
10X Buffer O	2 μ l
DNA (0.5-1 μ g/ μ l)	1 μ l
PagI	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 O	2 μ l
PagI	1-2 μ l
- 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 Fermentas 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 μ g 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 μ g of agarose-embedded lambda DNA in 16 hours.

Compatible Ends

AflIII, PstI, DsaI, Eco130I, NcoI

Number of Recognition Sites in DNA

λ	Φ X174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
8	3	4	3	3	2	1

Note

Low salt, high glycerol (>5%) concentrations, pH>8.0 or a large excess of PagI may result in star activity.

QUALITY CONTROL ASSAY DATA

Overdigestion Assay

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

Ligation/Recutting Assay

After a 50-fold overdigestion (3 u/μg DNA x 17 hours) with PstI, more than 95% 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 PstI for 4 hours.

Quality authorized by:



Jurgita Zilinskiene

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Certain countries are out of the scope of patent coverage.

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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.

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