

PRODUCT INFORMATION

Pael (SphI)

Lot: _____ Expiry Date: _____

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

Concentration: 10 u/μl
Source: *Pseudomonas aeruginosa*
Supplied with: 1 ml of 10X Buffer B
1 ml of 10X Buffer Tango

Store at -20°C



BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Buffer B (for 100% Pael digestion)

10 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 0.1 mg/ml BSA.

Incubation temperature

37°C.

Unit Definition

One unit is defined as the amount of Pael 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

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.

Storage Buffer

Pael is supplied in: 10 mM potassium-phosphate (pH 7.0 at 25°C), 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.15% Triton X-100, 0.5 mg/ml BSA and 50% glycerol.

Recommended Protocol for Digestion

- Add:

nuclease-free water	16 μ l
10X Buffer B	2 μ l
DNA (0.5-1 μ g/ μ l)	1 μ l
Pael	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 B	2 μ l
Pael	1-2 μ l
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours.

Thermal Inactivation

Pael is inactivated by incubation at 65°C for 20 min.

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

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

Methylation Effects on Digestion

Dam: never overlaps – no effect.

Dcm: never overlaps – no effect.

CpG: may overlap – no effect.

EcoKI: never overlaps – no effect.

EcoBI: may overlap – blocked.

Stability during Prolonged Incubation

A minimum of 0.1 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

Hin1II, Xcel

Number of Recognition Sites in DNA

λ	Φ X174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
6	0	1	1	1	1	1

CERTIFICATE OF ANALYSIS

Overdigestion Assay

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

Ligation/Recutting Assay

After a 50-fold overdigestion (3 u/µg DNA x 17 hours) with Pael, more than 95% of the digested DNA fragments can be ligated at a 5'-termini concentration of 0.06 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 was observed after the incubation with 10 units of Pael for 4 hours.

Blue/White Cloning Assay

pUC57 was incubated with 10 units of Pael for 16 hours. After religation and transformation the background level of white colonies was <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.thermoscientific.com/onebio for Material Safety Data Sheet of the product.

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