

PRODUCT INFORMATION

PfoI

#ER1751 200 U

Lot: ____ **Expiry Date:** __

5'...T↓C C N G G A...3'

3'...A G G N C C↑T...5'

Concentration: 10 U/μL

Source: *Pseudomonas fluorescens biovar 126*

Supplied with: 1 mL of 10X Buffer Tango

Store at -20°C



BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Thermo Scientific Tango Buffer (for 100% PfoI digestion)

33 mM Tris-acetate (pH 7.9), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/mL BSA.

Incubation temperature

37°C.

Unit Definition

One unit is defined as the amount of PfoI required to digest 1 μg of lambda DNA *dam*⁻, *dcm*⁻ 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

Tango™ Buffer provided simplifies buffer selection for double digests. 98% of Thermo Scientific restriction enzymes are active in a 1X or 2X concentration of Tango Buffer. Please go to www.thermoscientific.com/doubledigest to choose the best buffer for your experiments.

Storage Buffer

PfoI is supplied in: 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.

Note. Hydrolysis of one of the fifteen PfoI sites in phage lambda DNA (position 22349) proved approx. 50-times slower in comparison with the other sites.

Recommended Protocol for Digestion

- Add:

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

Thermal Inactivation

PfoI 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
0-20	20-50	50-100	0-20	100	50-100

Methylation Effects on Digestion

Dam: may overlap – cleavage impaired.

Dcm: may overlap – blocked.

CpG: may overlap – blocked.

EcoKI: never overlaps – no effect.

EcoBI: never overlaps – no effect.

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 10 units of the enzyme is required for complete digestion of 1 μ g of agarose-embedded lambda DNA in 16 hours.

Compatible Ends

EcoRII, SexAI, BssKI

Number of Recognition Sites in DNA

λ	Φ X174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
15	0	1	1	1	0	0

Note

PfoI cleavage is impaired by overlapping *dam* methylation and blocked by overlapping *dcm* methylation. To avoid *dam* or *dcm* methylation, use a *dam*⁻, *dcm*⁻ strain such as GM2163 (#M0099).

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion with PfoI (10 U/ μ g lambda DNA *dam*⁻, *dcm*⁻ 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 PfoI 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.

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