

PRODUCT INFORMATION

Pvul

#ER0621 300 U

Lot: ___ Expiry Date: _

5'...**C G A T↓C G**...3'

3'...**G C**↑ **T A G C**...5'

Concentration: 10 U/µL

Supplied with: 1 mL of 10X Buffer R

1 mL of 10X Buffer Tango

Store at -20°C

 $\mathsf{R} \hspace{0.1cm} \boxed{ 37^{0} \hspace{0.1cm} } \hspace{0.1cm} \boxed{ }$

CG







85

BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Buffer R (for 100% Pvul digestion) 10 mM Tris-HCl (pH 8.5), 10 mM MgCl₂, 100 mM KCl, 0.1 mg/mL BSA.

Incubation temperature

37°C.

Unit Definition

One unit is defined as the amount of Pvul required to digest 1 μ g lambda DNA-Cpol fragments in 1 hour at 37°C in 50 μ L 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.

Rev.11

Storage Buffer

Pvul is supplied in: 10 mM Tris-HCl (pH 7.5 at 25°C), 300 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.2 mg/mL BSA and 50% glycerol.

Recommended Protocol for Digestion

• Add:

nuclease-free water $16~\mu L$ 10X~Buffer~R $2~\mu L$ $DNA~(0.5-1~\mu g/\mu L)$ $1~\mu L$ Pvul $0.5-2~\mu L$

- 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 R 2 μ L Pvul 1-2 μ L

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

Thermal Inactivation

Only small amounts of Pvul (up to 10 units) can be inactivated by incubation at 80°C in 20 min.

Inactivation Procedure

- To prepare the digested DNA for electrophoresis:
 - stop the digestion reaction by adding 0.5 M EDTA, pH 8.0 (#R1021), to achieve a 20 mM final concentration. Mix thoroughly, add an electrophoresis loading dye and load onto gel.
- To prepare DNA suitable for further enzymatic reactions:
 - extract with phenol/chloroform, precipitate with ethanol or isopropanol, wash the pellet with 75% cold ethanol and air-dry;
 - dissolve DNA in either nuclease-free water, TE buffer, or a buffer suitable for further applications;
 - check the DNA concentration in the solution.

For **ENZYME PROPERTIES** and **CERTIFICATE OF ANALYSIS** see back page

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

В	G	0	R	Tango	2X Tango
0-20	20-50	50-100	100	50-100	100

Methylation Effects on Digestion

Dam: completely overlaps – no effect.

Dcm: never overlaps — no effect. CpG: completely overlaps — blocked.

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 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 µg of agarose-embedded lambda DNA in 16 hours.

Compatible Ends

Bsh1285I, SfaAI, PacI, BstKTI.

Number of Recognition Sites in DNA

λ	ФХ174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
3	0	1	2	2	2	1

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is after a 160-fold overdigestion with PvuI (10 U/ μ g lambda DNA \times 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 doublestranded labeled oligonucleotides occurred during incubation with 10 units of Pvul 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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