

CERTIFICATE OF ANALYSIS

# PpiI

#ER1541      50 u

Lot:                      Expiry Date:

5'...↓<sub>7</sub>( N ) G A A C ( N )<sub>5</sub> C T C ( N )<sub>13</sub>↓...3'  
3'...↑<sub>12</sub>( N ) C T T G ( N )<sub>5</sub> G A G ( N )<sub>8</sub> ↑...5'

Concentration:      2 u/μl  
Source:                *Pseudomonas putida* Jo 4-731  
Supplied with:      1 ml of 10X Buffer R  
                             1 ml of 10X Buffer Tango™

Store at -20°C



In total 3 vials.

BSA included: Lot# BSA62-313P

## RECOMMENDATIONS

**1X Buffer R** (for 100% PpiI digestion)

10 mM Tris-HCl (pH 8.5), 10 mM MgCl<sub>2</sub>, 100 mM KCl,  
0.1 mg/ml BSA.

**Incubation temperature**

30°C\*.

**Unit Definition**

One unit is defined as the amount of PpiI required to digest 1 μg of lambda DNA in 1 hour at 30°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 is provided to simplify buffer selection for double digests. 98% of Fermentas restriction enzymes are active in a 1X or 2X concentration of Tango™ Buffer.

Please refer to the Fermentas Catalog or go to [www.fermentas.com/doubledigest](http://www.fermentas.com/doubledigest) to choose the best buffer for your experiments.

1X Tango™ Buffer:

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

\* Incubation at 37°C results in 60% activity.

## Storage Buffer

Ppil is supplied in: 10 mM Tris-HCl (pH 7.4 at 25°C), 100 mM KCl, 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 R	2 µl
DNA (0.5-1 µg/µl)	1 µl
Ppil	0.5-2 µl**
- Mix gently and spin down for a few seconds.
- Incubate at 30°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 R	2 µl
Ppil	1-2 µl**
- Mix gently and spin down for a few seconds.
- Incubate at 30°C for 1-16 hours\*\*.

\*\* See Star Activity.

## Thermal Inactivation

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

## ENZYME PROPERTIES

### Enzyme Activity in Fermentas REase Buffers, %

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

### Star Activity

An excess of Ppil (6 u/µg lambda DNA x 2 hours) may result in star activity.

### Methylation Effects on Digestion

Dam: never overlaps – no effect.

Dcm: never overlaps – no effect.

CpG: may overlap – cleavage impaired.

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 µg of lambda DNA in 16 hours at 30°C.

### Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
5	0	2	2	2	3	1

### Note

Ppil cleaves certain DNA sequences at random 7 or 8nt away on the top strand of the recognition sequence:

5'...↓<sub>7-8</sub>(N)GAAC(N)<sub>5</sub>CTC(N)<sub>13</sub>↓...3'

3'...↑<sub>12</sub>(N)CTTG(N)<sub>5</sub>GAG(N)<sub>8</sub>↑...3'

The presence of SAM in a reaction mixture results in incomplete cleavage with Ppil.

For **QUALITY CONTROL ASSAY DATA** see back page

## QUALITY CONTROL ASSAY DATA

### Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 10-fold overdigestion with Ppil (5 u/μg lambda DNA x 2 hours) (see Star Activity).

### Ligation/Recutting Assay

After a 10-fold overdigestion (0.6 u/μg DNA x 17 hours) with Ppil, more than 90% of the digested DNA fragments can be ligated at a 5'-termini concentration of 0.05 μM. More than 90% 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 5 units of Ppil 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.fermentas.com](http://www.fermentas.com) for Material Safety Data Sheet of the product.