Thermo SCIENTIFIC

**PRODUCT INFORMATION**

# Ppu21I (BsaAI)

**#ER1971** 500 U

**Lot: ___** **Expiry Date: _**

5'...**Y A C↓G T R**...3'
3'...**R T G↑C A Y**...5'

Concentration: 10 U/µL
Source: *E.coli* that carries the cloned *ppu21IR* gene from *Pseudomonas putida* RFL21
Supplied with: 1 mL of 10X Buffer Ppu21I

**Store at -20°C**


BSA included

**www.thermoscientific.com/onebio**

## RECOMMENDATIONS

**1X Buffer Ppu21I** (for 100% Ppu21I digestion)
10 mM Tris-HCl (pH 7.2 at 37°C), 3 mM $MgCl_2$, 150 mM NaCl, 0.1 mg/mL BSA.

**Incubation Temperature**
30°C*****.

**Unit Definition**
One unit is defined as the amount of Ppu21I required to digest 1 µg of lambda DNA in 1 hour at 30°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 www.thermoscientific.com/doubledigest to choose the best buffer for your experiments.

**Storage Buffer**
Ppu21I is supplied in: 10 mM potassium phosphate (pH 7.4 at 25°C), 100 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.2 mg/mL BSA and 50% glycerol.

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***** Incubation at 37°C results in less than 30% activity.

## Recommended Protocol for Digestion

- Add:

| | |
|---|---|
| nuclease-free water | 16 µL |
| 10X Buffer Ppu21I | 2 µL |
| DNA (0.5-1 µg/µL) | 1 µL |
| Ppu21I | 0.5-1 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) |
| Water, nuclease-free | 18 µL |
| 10X Buffer Ppu21I | 2 µL |
| Ppu21I | 1-2 µL |

- Mix gently and spin down for a few seconds.
- Incubate at 30°C for 1-16 hours.

## Thermal Inactivation

Ppu21I is inactivated by incubation at 65°C for 20 min.

# ENZYME PROPERTIES

## Enzyme Activity in Thermo Scientific REase Buffers, %

| Ppu21I | B | G | O | R | Tango | 2X Tango |
|---|---|---|---|---|---|---|
| **100** | 50-100*** | 100*** | 20-50 | NR | NR | NR |

***– Star activity appears at a greater than 5-fold overdigestion (5 u x 1h).
NR – Buffer is not recommended, because of high star activity.

## Methylation Effects on Digestion

Dam: never overlaps – no effect.
Dcm: never overlaps – no effect.
CpG: completely overlaps – blocked.
EcoKI: may overlap – effect not determined.
EcoBI: may overlap – effect not determined.

## Stability during Prolonged Incubation

A minimum of 0.5 units of the enzyme is required for complete digestion of 1 µg of lambda DNA in 16 hours at 30°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.

## Number of Recognition Sites in DNA

| λ | ΦX174 | pBR322 | pUC57 | pUC18/19 | pTZ19R/U | M13mp18/19 |
|---|---|---|---|---|---|---|
| 14 | 2 | 1 | 0 | 0 | 1 | 5 |

For **CERTIFICATE OF ANALYSIS** *see* back page

# CERTIFICATE OF ANALYSIS

## Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion with Ppu21I (10 U/µg lambda DNA 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 Ppu21I for 4 hours.

## Blue/White (B/W) Cloning Assay

The B/W assay was replaced with LO test after validating experiments showed LO test ability to detect nuclease and phosphatase activities with sensitivity that equals to that of B/W test.

**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.