Thermo SCIENTIFIC

PRODUCT INFORMATION

PacI

#ER2202 1250 U

Lot: ___ **Expiry Date: _**

5'...**T T A A T↓T A A**...3'
3'...**A A T↑T A A T T**...5'

Concentration: 10 U/µL
Source: *E.coli* that carries the cloned *pacIR* gene from *Pseudomonas alcaligenes*
Supplied with: 1 mL of 10X Buffer PacI

Store at -20°C

BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Buffer PacI (for 100% PacI digestion)
10 mM Bis-Tris Propane-HCl (pH 6.5), 10 mM $MgCl_2$, 0.1 mg/mL BSA.

Incubation temperature
37°C.

Unit Definition
One unit is defined as the amount of PacI required to digest 1 µg of control DNA in 1 hour at 37°C in 50 µL of recommended reaction buffer. The control DNA is linearized pJET1 DNA with inserted PacI recognition site.

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.

Storage Buffer
PacI is supplied in: 10 mM potassium phosphate (pH 7.5 at 25°C), 200 mM NaCl, 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 PacI	2 µL
DNA (0.5-1 µg/µL)	1 µL
PacI	0.5-2 µL

- 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 µL (~0.1-0.5 µg of DNA)
nuclease-free water	18 µL
10X Buffer PacI	2 µL
PacI	1-2 µL

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

Thermal Inactivation

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

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

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

Methylation Effects on Digestion

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

Stability during Prolonged Incubation

A minimum of 0.3 unit of the enzyme is required for complete digestion of 1 µg of 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 pJET1 DNA with inserted PacI recognition sequence in 16 hours.

Compatible Ends

Bsh1285I, BstKTI, SfaNI (AsiSI), PvuI.

Number of Recognition Sites in DNA

Ad2	λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
1	0	0	0	0	0	0	1

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 PacI (10 U/µg 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 PacI 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.