



## CERTIFICATE OF ANALYSIS

# Van91I (PflMI)

#ER0712      2000 u

Lot:                      Expiry Date:

5'...C C A N N N N↓N T G G...3'  
3'...G G T N↑N N N N A C C...5'

Concentration:      10 u/μl  
Source:                *Vibrio anguillarum* RFL91  
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% Van91I digestion)

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

**Incubation temperature**

37°C.

**Unit Definition**

One unit is defined as the amount of Van91I required to digest 1 μg of lambda DNA 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 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.

## Storage Buffer

Van91I 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 $\mu$ l
10X Buffer R	2 $\mu$ l
DNA (0.5-1 $\mu$ g/ $\mu$ l)	1 $\mu$ l
Van91I	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 $\mu$ l (~0.1-0.5 $\mu$ g of DNA)
nuclease-free water	18 $\mu$ l
10X Buffer R	2 $\mu$ l
Van91I	1-2 $\mu$ l
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours.

## Thermal Inactivation

Van91I 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	50-100	50-100	100	20-50	50-100

## Methylation Effects on Digestion

Dam: never overlaps – no effect.

Dcm: may overlap – blocked.

CpG: never overlaps – no effect.

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  $\mu$ 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  $\mu$ g of agarose-embedded lambda DNA in 16 hours.

## Number of Recognition Sites in DNA

$\lambda$	$\Phi$ X174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
14	2	2	0	0	0	0

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 160-fold overdigestion with Van 91I (10 u/μg lambda DNA x 16 hours).

## Ligation/Recutting Assay

After a 50-fold overdigestion (3 u/μg DNA x 17 hours) with Van 91I, more than 90% of the digested DNA fragments can be ligated at a 5'-termini concentration of 0.1 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 10 units of Van 91I for 4 hours.

## Blue/White Cloning Assay

A mixture of pUC57/HindIII, pUC57/Eco32I and pUC57/PstI digests was incubated with 10 units of Van 91I for 16 hours. After religation and transformation, the background level of white colonies was 0.3%.

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.