

CERTIFICATE OF ANALYSIS

# Kpn2I (BspEI)

#ER0532      2500 u

Lot:                      Expiry Date:

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

Concentration:      10 u/μl  
Source:                *E.coli* that carries the cloned *kpn2IR*  
gene from *Klebsiella pneumoniae*  
RFL2

Supplied with:      1 ml of 10X Buffer Tango™

Store at -20°C



In total 2 vials.

BSA included: Lot# BSA62-313P

## RECOMMENDATIONS

**1X Buffer Tango™** (for 100% Kpn2I digestion)  
33 mM Tris-acetate (pH 7.9), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/ml BSA.

### Incubation temperature

55°C\*.

### Unit Definition

One unit is defined as the amount of Kpn2I required to digest 1 μg of lambda DNA in 1 hour at 55°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.

### Storage Buffer

Kpn2I is supplied in: 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.

\* Incubation at 37°C results in 50% activity.

## Recommended Protocol for Digestion

- Add:

nuclease-free water	16 $\mu$ l
10X Buffer Tango™	2 $\mu$ l
DNA (0.5-1 $\mu$ g/ $\mu$ l)	1 $\mu$ l
Kpn2I	0.5-2 $\mu$ l
- Mix gently and spin down for a few seconds.
- Incubate at 55°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 Tango™	2 $\mu$ l	
Kpn2I	1-2 $\mu$ l	
- Mix gently and spin down for a few seconds.
- Incubate at 55°C for 1-16 hours.

## Thermal Inactivation

Kpn2I is inactivated by incubation at 80°C for 20 min.

## ENZYME PROPERTIES

### Enzyme Activity in Fermentas REase Buffers, %

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

### Methylation Effects on Digestion

Dam: may overlap – 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 0.1 units of the enzyme is required for complete digestion of 1  $\mu$ g of lambda DNA in 16 hours at 55°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.

### Compatible Ends

BshTI, BsaWI, Cfr9I, Cfr10I, Eco88I, NgoMIV, SgrAI

### Number of Recognition Sites in DNA

$\lambda$	$\Phi$ X174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
24	0	1	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 Kpn2I (10 u/μg lambda DNA x 16 hours).

## Ligation/Recutting Assay

After a 50-fold overdigestion (3 u/μg DNA x 17 hours) with Kpn2I, more than 95% of the digested DNA fragments can be ligated at a 5'-termini concentration of 0.3 μM. More than 95% 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 Kpn2I for 4 hours.

## Blue/White Cloning Assay

A mixture of pUC57/HindIII, pUC57/Eco32I and pUC57/PstI digests was incubated with 10 units of Kpn2I for 16 hours. After religation and transformation, the background level of white colonies was 0.1%.

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.