Fermentas
LIFE SCIENCES

**CERTIFICATE OF ANALYSIS**

# **Kpn2I** (BspEI)

**#ER0531** 500 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

ISO 9001 ISO 14001
www.fermentas.com

## 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 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.

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

## Recommended Protocol for Digestion

- Add:

| | |
|---|---|
| nuclease-free water | 16 µl |
| 10X Buffer Tango™ | 2 µl |
| DNA (0.5-1 µg/µl) | 1 µl |
| Kpn2I | 0.5-2 µl |

- 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 µl | (~0.1-0.5 µg of DNA) |
| nuclease-free water | 18 µl | |
| 10X Buffer Tango™ | 2 µl | |
| Kpn2I | 1-2 µl | |

- 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 µg 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 µg of agarose-embedded lambda DNA in 16 hours.

## Compatible Ends

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

## Number of Recognition Sites in DNA

| λ | ΦX174 | 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 for Material Safety Data Sheet of the product.