Kpn I

Cat#: orb93994 (Product Manual)

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

Unit Definition: One unit is the amount of enzyme required to completely digest 1 µg of Lambda DNA (EcoRI digest, 2 sites) in 1 hour in a total reaction volume of 50 µl. Enzyme activity was determined in the recommended reaction buffer.

For in vitro use only!

Shipping: shipped on blue ice

Storage Conditions: store at -20 °C

Additional Storage Conditions: avoid freeze/thaw cycles

Shelf Life: 12 months

Form: liquid (Supplied in 10 mM Tris-HCl pH 7.4, 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 200 µg/ml BSA and 50 % [v/v] glycerol)

Concentration: 10 units/µl

Source: Klebsiella pneumoniae OK8

Supplied with: 10x Universal Buffer (UB)

Recommended 50 µl assay

5 µl	10x Universal Buffer (UB)
1 µg	pure DNA1 or PCR product2
5 units	enzyme
fill up to 50 µl	PCR grade water

1 Supercoiled or high molecular weight DNA (e.g. plant genomic DNA) may require longer incubation time or higher amount of enzyme.

2 Some enzymes may require additional DNA bases flanking the restriction site for complete digestion.

Protocol:

- The enzyme should not exceed 10 % of total reaction volume.
- Add enzyme as last component. Mix components well before adding enzyme. After enzyme addition, mix gently by pipetting. Do not vortex.
- Incubate 60 min. at 37 °C.
- Stop reaction by alternatively:

- Addition of 2.1 µl EDTA pH 8.0 [0.5 M], final 20 mM
- Heat Inactivation (No)
- Spin Column DNA Purification
- Gel Electrophoresis and Single Band Excision
- Phenol-Chloroform Extraction or Ethanol Precipitation.

Double Digestion - Buffer Compatibility:

B1 - 75-100 % Relative Activity

B2 - 25-50 % Relative Activity

B3 - <10 % Relative Activity

B4 - <10 % Relative Activity

B5 - 50 % Relative Activity

1x UB - 100 % Relative Activity (recommended)

Please note that the optimum digestion condition for this en-zyme is 1x UB. Within the Universal Buffer (UB) system, the most majority of our enzymes display 100% Relative Activity in 1x UB and only few either in 0.5x or 2x UB. If optimum condition for second enzyme is different than the recommended for the first enzyme, we suggest carrying out first the restriction at the higher recommended concentration of UB and dilute the reaction volume to the adequate UB concentration for further proceeding with the second restriction

Reaction Enzymes Buffer Guide:

Buffer 1	10×B1	100 mM 100 mM 1000 µg/ml	Tris-HCl (pH 7.9, 25°C) MgCl2 BSA
Buffer 2	10×B2	100 mM 100 mM 500 mM 1000 µg/ml	Tris-HCl (pH 7.9, 25°C) MgCl2 NaCl BSA
Buffer 3	10×B3	500 mM 100 mM 1000 mM 1000 µg/ml	Tris-HCl (pH 7.9, 25°C) MgCl2 NaCl BSA
Buffer 4	10×B4	100 mM 100 mM 1500 mM 1000 µg/ml	Tris-HCl (pH 7.9, 25°C) MgCl2 NaCl BSA
Buffer 5	10×B5	200 mM 100 mM 500 mM 1000 µg/ml	Tris-acetate (pH 7.9, 25°C) Mg-acetate K-acetate BSA

Reaction Buffer Compatibility:

Our restriction enzymes are fully compatible to restrictases and buffer systems from other manufacturers and can be used along in double digestions. To obtain best results, consult the corresponding manuals of all involved products.

Ligation and recutting:

After 10-fold overdigestion with KpnI, >95% of the DNA fragments can be ligated and recut with this enzyme.

Star activity:

Conditions of low ionic strength, high enzyme concentration, glycerol concentration >5 % or pH >8.0 may result in star activity.

DNA Methylation:

No Inhibition: dcm, dam, CpG

Quality Control:

All preparations are assayed for contaminating endonuclease, 3'-exonuclease, 5' exonuclease/ 5' phosphatase, as well as nonspecific single- and doublestranded DNase activities.

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