KpnI

1-800-632-7799
info@neb.com
www.neb.com

R0142S 053120814081

R0142S

4,000 units **10,000 U/ml** **Lot: 0531208**
RECOMBINANT **Store at –20°C** **Exp: 8/14**

Recognition Site:

5′. . . G G T A C▼C . . . 3′
3′. . . C▲C A T G G . . . 5′

Source: An *E. coli* strain that carries the cloned KpnI gene from *Klebsiella pneumoniae* OK8 (ATCC 49790)

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

Reagents Supplied with Enzyme:
10X NEBuffer 1, 100X BSA.

Reaction Conditions: 1X NEBuffer 1, supplemented with 100 µg/ml BSA. Incubate at 37°C.

1X NEBuffer 1:
10 mM Bis Tris Propane-HCl
10 mM $MgCl_2$
1 mM DTT
pH 7.0 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of Adenovirus-2 DNA in 1 hour at 37°C in a total reaction volume of 50 µl.

Diluent Compatibility: Diluent Buffer A
50 mM KCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 200 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C)

Quality Control Assays

Ligation: After 50-fold overdigestion with KpnI, > 95% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5′ termini concentration of 1–2 µM) at 16°C. Of these ligated fragments, > 95% can be recut.

16-Hour Incubation: A 50 µl reaction containing 1 µg of DNA and 60 units of enzyme incubated for 16 hours resulted in the same pattern of DNA bands as a reaction incubated for 1 hour with 1 unit of enzyme.

Exonuclease Activity: Incubation of 300 units of enzyme with 1 µg sonicated ^3H DNA (10^5 cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

Endonuclease Activity: Incubation of 30 units of enzyme with 1 µg of ϕX174 RF I DNA for 4 hours at 37°C in 50 µl reaction buffer resulted in <10% conversion to RF II.

Blue/White Screening Assay: This enzyme has been tested to determine the integrity of the DNA ends produced after digestion with an excess of enzyme. An appropriate vector is digested at a unique site within *lacZ*$^\alpha$ gene with a 10-fold excess of enzyme, ligated, transformed and plated on XGal/IPTG/Amp plates. Successful expression of β-galactosidase is a function of how intact its gene remains after cloning, an intact gene gives rise to a blue colony, an interrupted gene (i.e. degraded DNA end) gives rise to a white colony. Enzymes must produce fewer than 3% white colonies to be Blue/White Certified.

Enzyme Properties

Activity in NEBuffers:

NEBuffer 1	**100%**
NEBuffer 2	75%
NEBuffer 3	0%
NEBuffer 4	50%

When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.

(see other side)

CERTIFICATE OF ANALYSIS

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(see other side)

CERTIFICATE OF ANALYSIS

Survival in a Reaction: A minimum of 0.25 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: No

Plasmid Cleavage: Number of units required to cleave 1 µg of supercoiled plasmid DNA in one hour: pUC19 = 2 units.

Notes: Acc 65I is an isoschizomer of KpnI. KpnI produces a 4-base 3´ extension, whereas Acc 65I produces a 4-base 5´ extension.

Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Companion Products Sold Separately:

KpnI-HF™

#R3142S	4,000 units
#R3142L	20,000 units
#R3142M	20,000 units

KpnI-HF™ RE-Mix™

#R5142S	200 reactions

= Time-Saver™ Qualified (See www.neb.com for details).

U.S. Patent Nos. 5,082,784; 5,192,675

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