KpnI-HF™

NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

R3142S 003120814081

R3142S

RR e 37° No

4,000 units	**20,000 U/ml**	**Lot: 0031208**
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′

Note: KpnI-HF™ has the same specificity as KpnI (NEB #R0142), but it has been engineered for reduced star activity.

Source: An *E. coli* strain that carries the cloned and modified 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 4.

Reaction Conditions: 1X NEBuffer 4.
Incubate at 37°C.

1X NEBuffer 4:
50 mM potassium acetate
20 mM Tris-acetate
10 mM magnesium acetate
1 mM DTT
pH 7.9 at 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of pXba 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 Controls

Ligation: After 50-fold overdigestion with KpnI-HF, > 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 200 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 400 units of enzyme with 1 µg sonicated ^{3}H DNA (10^{5} cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

Endonuclease Activity: Incubation of 100 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	25%
NEBuffer 3	0%
NEBuffer 4	**100%**

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

Survival in a Reaction: A minimum of 0.5 unit is required to digest 1 µg of substrate DNA in 16 hours.

Heat Inactivation: No

(See other side)

CERTIFICATE OF ANALYSIS

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(See other side)

CERTIFICATE OF ANALYSIS

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

Notes: Acc65I 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

#R0142S	4,000 units
#R0142L	20,000 units
#R0142M	20,000 units

KpnI-HF™ RE-Mix™

#R5142S	200 reactions

New icons (see www.neb.com for details)

= Time-Saver™ Qualified

= indicates that the enzyme has been engineered

= indicates that the enzyme has reduced star activity

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