KasI

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

R0544S 040121113111

R0544S

BSA 37° NEB4 Yes

250 units **4,000 U/ml** **Lot: 0401211**

RECOMBINANT **Store at –20°C** **Exp: 11/13**

Recognition Site:

5′. . . G▼GCGCC . . . 3′
3′. . . CCGCG▲G . . . 5′

Source: An *E. coli* strain that carries the cloned KasI gene from *Kluyvera ascorbata* (C. Polisson)

New Reaction Buffer

Supplied in: 500 mM KCl, 20 mM Tris-HCl (pH 7.0), 0.1 mM EDTA, 0.1% Triton X-100, 1 mM $MgCl_2$, 200 µg/ml BSA and 50% glycerol.

Note: –80°C is recommended for storage longer than 6 months.

Reagents Supplied with Enzyme:
10X NEBuffer 4, 100X BSA.

Reaction Conditions: 1X NEBuffer 4, supplemented with 100 µg/ml BSA. 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 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of pBR322 DNA in 1 hour at 37°C in a total reaction volume of 50 µl.

Diluent Compatibility: Diluent Buffer B
300 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 500 µg/ml BSA and 50% glycerol (pH 7.4 @ 25°C)

Quality Control Assays

Ligation: After 20-fold overdigestion with KasI, > 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 4 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 10 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 0.8% radioactivity.

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	25%
NEBuffer 2	100%
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.

(See other side)

CERTIFICATE OF ANALYSIS

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(See other side)
CERTIFICATE OF ANALYSIS

Survival in a Reaction: Not recommended for digest over 1 hour.

Heat Inactivation: 20 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Notes: KasI is an isoschizomer of NarI and SfoI.

KasI produces a 4-base 5´ extension whereas NarI produces a 2-base 5´ extension. KasI demonstrates marked site preference and is 25-fold more active on λ DNA than on pBR322 DNA.

Cleavage of mammalian genomic DNA is blocked by CpG methylation.

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