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R0554S MEA CO BSA 60° MM

Lot: 0061208 Exp: 8/14 1.000 units 10.000 U/ml Store at -20°C

Recognition Site:

5′... C G R Y C G ... 3′ 3′... G C₄Y R G C ... 5′

Single Letter Code: R = A or G, Y = C or T

Source: Bacillus species (D. Clark)

New Reaction Buffer

BsiEI info@neb.com www.neb.com R0554S NEA CO BSA 60° WW

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5′... C G R Y C G ... 3′ 3′... G C,Y R G C ... 5′

Single Letter Code: R = A or G. Y = C or T

Source: Bacillus species (D. Clark)

New Reaction Buffer

Supplied in: 50 mM NaCl, 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, 100X BSA.

Reaction Conditions: 1X NEBuffer 4, supplemented with 100 µg/ml BSA. Incubate at 60°C.

1X NEBuffer 4:

50 mM potassium acetate 20 mM Tris-acetate 10 mM magnesium acetate 1 mM dithiothreitol pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of λ DNA in 1 hour at 60°C in a total reaction volume of 50 µl.

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

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Quality Control Assays

Ligation: After 10-fold overdigestion with BsiEl. > 95% of the DNA fragments can be ligated with T4 DNA Ligase (at a 5' termini concentration of $1-2 \mu$ M) at 16°C. Of these ligated fragments, > 95% can be recut.

16-Hour Incubation: A 50 µl reaction containing 1 µg of DNA and 100 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 100 units of enzyme with 1 µg sonicated 3H DNA $(10^5 \text{ cpm/}\mu\text{g})$ for 4 hours at 60°C in 50 μI reaction buffer released < 0.1% radioactivity

Enzyme Properties

Activity in NEBuffers: NEBuffer 1 50%

NEBuffer 2 100% NEBuffer 3 10% 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: Intermediate activity. Suitable for extended digestion, but < 8 hours.

Heat Inactivation: 80°C for 20 minutes.

Note: Cleavage of mammalian genomic DNA is blocked by CpG methylation.

Incubation at 37°C results in 30% activity.

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CERTIFICATE OF ANALYSIS

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NEBuffer 1 50% NEBuffer 2 100% 10% NEBuffer 3

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Image: Saver[™] Qualified (See www.neb.com for details).

