hSMUG1

NEW ENGLAND BioLabs Inc.
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M0336S 001150116011

M0336S

RR BSA 37° Yes

500 units **5,000 U/ml** **Lot: 0011501**

RECOMBINANT **Store at –20°** **Exp: 1/16**

Description: Human single-strand-selective monofunctional uracil-DNA Glycosylase SMUG1 excises deoxyuracil and deoxyuracil-derivatives bearing an oxidized group at C5, such as 5-hydroxyuracil, 5-hydroxymethyluracil and 5-formyluracil in ssDNA and dsDNA (1,2,3).

Source: An *E. coli* strain which carries the cloned human SMUG1 gene.

Applications:

- Oxidative DNA damage studies
- Single cell gel electrophoresis (comet assay) (4,5,6)

Supplied in: 250 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 0.15% Triton X-100, 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 excize 1 pmol of deoxyuracil from a 34 mer oligonucleotide duplex containing a single dU site in a total reaction volume of 10 µl in 1 hour at 37°C.

Molecular Weight: 29,861 Daltons

Diluent Compatibility: Diluent C
250 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 0.15% Triton X-100, 200 µg/ml BSA and 50% glycerol.

Unit Assay Conditions: 1X NEBuffer 1 supplemented with 100 µg/ml BSA containing 5 pmol of fluorescently labeled oligonucleotide duplex in a total reaction volume of 10 µl. After release of dU, the oligonucleotide is cleaved by treating the resulting AP site with 100 mM NaOH for 10 minutes at 80°C.

Quality Control Assays

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection. BSA is added to the enzyme for stability.

16-Hour Incubation: A 50 µl reaction containing 1 µg of λ DNA (HindIII digest) and 50 units of hSMUG1 incubated for 16 hours at 37°C resulted in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Exonuclease Activity: Incubation of a 20 ul reaction containing 50 units of hSMUG1 with a 10 nM mixture of single-stranded and double-stranded fluorescent-labeled oligonucleotides containing blunt ends, 5' extensions, and 3' extensions for 30 minutes at 37°C yields no detectable degradation as determined by high resolution capillary electrophoresis.

Endonuclease Activity: Incubation of a 50 µl reaction containing 50 units of hSMUG1 with 1 µg ϕX174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RFII as determined by agarose gel electrophoresis.

Heat Inactivation: 20 minutes at 65°C.

Usage Note: hSMUG1 has 50% activity on 5-hydroxymethyluracil when compared to uracil. hSMUG1 has 50% activity on ssDNA compared to ds DNA.

References:

1. Cannon-Crlson, S.V. et al. (1989) *J. Biol. Chem.* 264, 13306-13312.
2. Masaoka, A. et al. (2003) *Biochemistry* 42, 5003-5012.
3. Wibley, J.E.A. et al. (2003) *Cell* 11, 1647-1659.
4. Singh, N. et al. (1988) *Experimental Cell Research* 175, 184–191.
5. Collins, A. et al. (1993) *Carcinogenesis* 14, 1733–1735.
6. Collins, A. et al. (1996) *Environmental Health Perspectives* 104, 465–469.

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