

# hSMUG1



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M0336S 001150716071

**M0336S**

**500 units**    **5,000 U/ml**    **Lot: 0011507**

**RECOMBINANT**    **Store at -20°**    **Exp: 7/16**

**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)

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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<sub>2</sub>

1 mM DTT

pH 7.0 @ 25°C

**Unit Definition:** One unit is defined as the amount of enzyme required to excise 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.

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**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 µl reaction containing 50 units of hSMUG1 with a 10 nM mixture of single-stranded and double-stranded fluorescently 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

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containing 50 units of hSMUG1 with 1 µg φX174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RFI 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.

CERTIFICATE OF ANALYSIS

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