

Afu Uracil-DNA Glycosylase (UDG)



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M0279S 003150717071

M0279S



200 units 2,000 U/ml Lot: 0031507

RECOMBINANT Store at -20°C Exp: 7/17

Description: A thermostable homolog of the *E. coli* Uracil-DNA Glycosylase (UDG) (1,2) from *Archaeoglobus fulgidus*. *Afu* UDG catalyzes the release of free uracil from uracil-containing DNA. *Afu* UDG efficiently hydrolyzes uracil from single-stranded or double-stranded DNA.

Source: Cloned and overexpressed in *E. coli*.

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4 @ 25°C), 0.1 mM EDTA, 1 mM DTT, 0.1 mg/ml BSA and 50% glycerol.

New Reaction Buffer

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Reagents Supplied with Enzyme:
10X ThermoPol II (Mg-free) Reaction Buffer.

Reaction Conditions: 1X ThermoPol II (Mg-free) Reaction Buffer. Incubate at 65°C.

1X ThermoPol II (Mg-free) Reaction Buffer:
20 mM Tris-HCl
10 mM KCl
10 mM (NH₄)₂SO₄
0.1% Triton X-100
(pH 8.8 @ 25°C).

Unit Definition: One unit is defined as the amount of enzyme that catalyzes the release of 60 pmol of uracil per minute from double-stranded, uracil-containing DNA. Activity is measured by release of [³H]-uracil in a 50 µl reaction containing 0.2 µg DNA (10⁴-10⁵ cpm/µg) in 30 minutes at 65°C.

Unit Assay Conditions: 1X ThermoPol II (Mg-free) Reaction Buffer, 1 unit of *Afu* Uracil-DNA Glycosylase, 0.2 µg ³H-uracil DNA (10⁴-10⁵ cpm/µg) for 30 minutes at 65°C in a total reaction volume of 50 µl.

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Molecular Weight: 22,717 daltons.

Specific Activity: 96 units/µg

Quality Control Assays

16-Hour Incubation: A 50 µl reaction containing 1 µg of λ phage DNA in ThermoPol Reaction Buffer and 50 units of *Afu* UDG showed no degradation following overnight incubation at 65°C.

Exonuclease Activity: Incubation of 50 units for 4 hours at 65°C in 50 µl of ThermoPol Reaction Buffer with 1 µg ³H DNA (10⁵ cpm/µg) released < 0.1% radioactivity.

Endonuclease Activity: Incubation of 5 units of *Afu* UDG with 1 µg of φX174 RF I DNA for 4 hours at 65°C in 50 µl ThermoPol Reaction Buffer resulted in < 20% conversion to RF II.

Heat Inactivation: No

Note: *Afu* UDG retains 50% activity in the presence of 150 mM NaCl. *Afu* UDG retains less than 1% activity after boiling for 30 minutes in standard reaction conditions.

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Under standard reaction conditions, uracil glycosylase inhibitor (UGI) does not inhibit *Afu* UDG.

References:

1. Sandigursky, M. and Franklin, W. A. (1999) *Current Biology* 9, 531-534.
2. Sandigursky, M. and Franklin, W. A. (2000) *J. Biol. Chem.* 275, 19146-19149.

CERTIFICATE OF ANALYSIS

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