New England Biolabs
Product Specification

Product Name: Afu Uracil-DNA Glycosylase (UDG)
Catalog #: M0279S/L
Concentration: 2,000 units/ml
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 $[^3]H$-uracil in a 50 µl reaction containing 0.2 µg DNA ($10^4$- $10^5$ cpm/µg) in 30 minutes at 65°C.

Shelf Life: 24 months
Storage Temp: Store between -25 and -15°C
Storage Conditions: 10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.1 mg/ml BSA, 50% Glycerol, pH 7.4 @ 25°C
Specification Version: PS-M0279S/L v2.0
Effective Date: 14 Feb 2014

Assay Name/Specification (minimum release criteria)

DNase Activity (Labeled Oligo, 3' extension) - A 50 µl reaction in CutSmart™ Buffer containing a 20 nM solution of a fluorescent labeled double-stranded oligonucleotide containing a 3’ extension and a minimum of 10 units of Afu Uracil-DNA Glycosylase (UDG) incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.

DNase Activity (Labeled Oligo, 5' extension) - A 50 µl reaction in CutSmart™ Buffer containing a 20 nM solution of a fluorescent labeled double-stranded oligonucleotide containing a 5’ extension and a minimum of 10 units of Afu Uracil-DNA Glycosylase (UDG) incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.

Double Stranded DNase Activity (Labeled Oligo) - A 50 µl reaction in CutSmart™ Buffer containing a 20 nM solution of a fluorescent labeled double-stranded oligonucleotide containing a blunt end and a minimum of 10 units of Afu Uracil-DNA Glycosylase (UDG) incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.

Endonuclease Activity (Nicking) - A 50 ul reaction in ThermoPol Reaction Buffer containing 1 ug of supercoiled PhiX174 DNA and a minimum of 4 units of Afu Uracil-DNA Glycosylase (UDG) incubated for 4 hours at 65°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.

Exonuclease Activity (Radioactivity Release) - A 50 µl reaction in ThermoPol Reaction Buffer containing 1 µg of a mixture of single and double-stranded $[^3]H$ E. coli DNA and a minimum of 50 units of Afu Uracil-DNA Glycosylase (UDG) incubated for 4 hours at 65°C releases <0.1% of the total radioactivity.
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<tr>
<th>Assay Name/Specification (minimum release criteria)</th>
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<tr>
<td><strong>Non-Specific DNase Activity (16 Hour)</strong> - A 50 ul reaction in ThermoPol Reaction Buffer containing 1 ug of Lambda DNA and a minimum of 50 units of Afu Uracil-DNA Glycosylase (UDG) incubated for 16 hours at 65°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.</td>
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<td><strong>Protein Concentration (A280/NanoDrop)</strong> - The concentration of Afu Uracil-DNA Glycosylase (UDG) is 0.027 mg/ml ± 10% as determined by UV absorption at 280 nm. Protein concentration is determined by the Pace method using the extinction coefficient of 16,110 and molecular weight of 22,720 daltons for Afu Uracil-DNA Glycosylase (UDG) (Pace, C.N. et al. (1995) Protein Sci., 4, 2411-2423).</td>
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<td><strong>Protein Purity Assay (SDS-PAGE)</strong> - Afu Uracil-DNA Glycosylase (UDG) is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</td>
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<td><strong>RNase Activity (Extended Digestion)</strong> - A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single stranded RNA and a minimum of 2 units of Afu Uracil-DNA Glycosylase (UDG) is incubated at 37°C. After incubation for 16 hours, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using polyacrylamide gel electrophoresis detection.</td>
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<td><strong>Single Stranded DNase Activity (FAM-Labeled Oligo)</strong> - A 50 µl reaction in CutSmart™ Buffer containing a 20 nM solution of a fluorescent internal labeled oligonucleotide and a minimum of 10 units of Afu Uracil-DNA Glycosylase (UDG) incubated for 16 hours at 37°C yields &lt;5% degradation as determined by capillary electrophoresis.</td>
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* The BSA in this product has been granted an EDQM "Certificate of Suitability" from the European Directorate for the Quality of Medicines (R1-CEP-2003-204-Rev00) and has been granted a USDA Certificate for Export of Bovine Blood Plasma/Serum for Manufacture into Pharmaceutical Products.

Derek Robinson  
Director of Quality Control