

New England Biolabs Product Specification

<i>Product Name:</i>	<i>Afu Uracil-DNA Glycosylase (UDG)</i>
<i>Catalog #:</i>	<i>M0279S/L</i>
<i>Concentration:</i>	<i>2,000 units/ml</i>
<i>Unit Definition:</i>	<i>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.</i>
<i>Shelf Life:</i>	<i>24 months</i>
<i>Storage Temp:</i>	<i>Store between -25 and -15 °C</i>
<i>Storage Conditions:</i>	<i>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</i>
<i>Specification Version:</i>	<i>PS-M0279S/L v1.0</i>
<i>Effective Date:</i>	<i>05 Dec 2013</i>

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 µg 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 [³H] *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.

Non-Specific DNase Activity (16 Hour) - A 50 ul reaction in ThermoPol Reaction Buffer containing 1 µg 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.

Protein Concentration (Bradford Method) - The concentration of Afu Uracil-DNA Glycosylase (UDG) is 0.028 mg/ml +/- 10% as determined by the Bradford Method using Bovine Serum Albumin as a standard.

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Protein Purity Assay (SDS-PAGE) - Afu Uracil-DNA Glycosylase (UDG) is $\geq 95\%$ pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

RNase Activity (Extended Digestion) - A 10 μ l 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, $>90\%$ of the substrate RNA remains intact as determined by gel electrophoresis using polyacrylamide gel electrophoresis detection.

Single Stranded DNase Activity (FAM-Labeled Oligo) - A 50 μ l 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 $<5\%$ degradation as determined by capillary electrophoresis.

** 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

Date 05 Dec 2013

