

## New England Biolabs Certificate of Analysis

*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 [<sup>3</sup>H]-uracil in a 50 µl reaction containing 0.2 µg DNA (10<sup>4</sup>-10<sup>5</sup> cpm/µg) in 30 minutes at 65°C.  
*Lot #:* 0031705  
*Assay Date:* 05/2017  
*Expiration Date:* 05/2019  
*Storage Temp:* -20°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 v3.0  
*Effective Date:* 26 Jul 2016

Assay Name/Specification (minimum release criteria)	Lot #0031705
<b>DNase Activity (Labeled Oligo, 3' extension)</b> - 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.	<b>Pass</b>
<b>DNase Activity (Labeled Oligo, 5' extension)</b> - 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.	<b>Pass</b>
<b>Double Stranded DNase Activity (Labeled Oligo)</b> - 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.	<b>Pass</b>
<b>Endonuclease Activity (Nicking)</b> - 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.	<b>Pass</b>



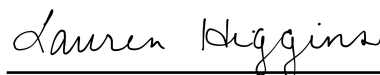
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Assay Name/Specification (minimum release criteria)	Lot #0031705
<b>Exonuclease Activity (Radioactivity Release)</b> - A 50 µl reaction in ThermoPol Reaction Buffer containing 1 µg of a mixture of single and double-stranded [ <sup>3</sup> H] <i>E. coli</i> 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.	<b>Pass</b>
<b>Non-Specific DNase Activity (16 Hour)</b> - 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.	<b>Pass</b>
<b>Protein Concentration (A280/NanoDrop)</b> - 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).	<b>Pass</b>
<b>Protein Purity Assay (SDS-PAGE)</b> - Afu Uracil-DNA Glycosylase (UDG) is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	<b>Pass</b>
<b>RNase Activity (Extended Digestion)</b> - 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, >90% of the substrate RNA remains intact as determined by gel electrophoresis using polyacrylamide gel electrophoresis detection.	<b>Pass</b>
<b>Single Stranded DNase Activity (FAM-Labeled Oligo)</b> - 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 <5% degradation as determined by capillary electrophoresis.	<b>Pass</b>

\* 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.



Authorized by  
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26 Jul 2016



Inspected by  
Lauren Higgins  
02 Jun 2017

