

## New England Biolabs Certificate of Analysis

**Product Name:** *Afu Uracil-DNA Glycosylase (UDG)*  
**Catalog Number:** *M0279S*  
**Concentration:** *2,000 U/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 (104-105 cpm/µg) in 30 minutes at 65°C.*  
**Lot Number:** *10047840*  
**Expiration Date:** *06/2021*  
**Storage Temperature:** *-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*

Afu Uracil-DNA Glycosylase (UDG) Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0279SVIAL	Afu Uracil-DNA Glycosylase (UDG)	10047841	Pass
B9005SVIAL	ThermoPol® II (Mg-free) Reaction Buffer Pack	0021803	Pass

Assay Name/Specification	Lot # 10047840
<p><b>Non-Specific DNase Activity (16 Hour)</b>            A 50 µl 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.</p>	Pass
<p><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] E. coli DNA and a minimum of 50 units of Afu Uracil-DNA Glycosylase (UDG) incubated for 4 hours at 65°C releases &lt;0.1% of the total radioactivity.</p>	Pass
<p><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 &lt;5%</p>	Pass

Assay Name/Specification	Lot # 10047840
degradation as determined by capillary electrophoresis.	
<p><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 &lt;5% degradation as determined by capillary electrophoresis.</p>	<b>Pass</b>
<p><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 &lt;5% degradation as determined by capillary electrophoresis.</p>	<b>Pass</b>
<p><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 &lt;5% degradation as determined by capillary electrophoresis.</p>	<b>Pass</b>
<p><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.</p>	<b>Pass</b>
<p><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, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using polyacrylamide gel electrophoresis detection.</p>	<b>Pass</b>
<p><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).</p>	<b>Pass</b>
<p><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 &lt;20% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	<b>Pass</b>

This product has been tested and shown to be in compliance with all specifications.

*Lauren Higgins*

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Lauren Sears Higgins  
Production Scientist  
27 Jun 2019

*Michael Tonello*

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Michael Tonello  
Packaging Quality Control Inspector  
01 Jul 2019