

New England Biolabs Product Specification

Product Name:	<i>Afu Uracil-DNA Glycosylase (UDG)</i>
Catalog #:	<i>M0279S</i>
Concentration:	<i>2,000 units/ml</i>
Unit Definition:	<i>One unit is defined as the amount of enzyme required to release 60 pmol per minute of a fluorescently labeled 47-mer single-stranded DNA oligonucleotide containing a single uracil base in 30 minutes at 65°C in a total reaction volume of 50 µl in 1X ThermoPol II Buffer.</i>
Shelf Life:	<i>24 months</i>
Storage Temp:	<i>-20°C</i>
Storage Conditions:	<i>10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.1 mg/ml rAlbumin, 50% Glycerol, pH 7.4 @ 25°C</i>
Specification Version:	<i>PS-M0279S v4.0</i>
Effective Date:	<i>12 Jan 2024</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 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 [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.



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Non-Specific DNase Activity (16 Hour) - A 50 μ l reaction in ThermoPol Reaction Buffer containing 1 μ g 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 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.

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Date 12 Jan 2024

Nancy Considine
Quality Approver

