

New England Biolabs Product Specification

Product Name:	WarmStart [®] Afu Uracil-DNA Glycosylase (UDG)
Catalog #:	M1282S
Concentration:	2,000 units/ml
Unit Definition:	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.
Shelf Life:	24 months
Storage Temp:	-20°C
Storage Conditions:	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)
Specification Version:	PS-M1282S v1.0
Effective Date:	14 Nov 2023

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 µl 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 rCutSmart[™] 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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Functional Testing (WarmStart Inhibition) - A 50 µl reaction in ThermoPol[®] II Buffer containing a minimum of 2 units of WarmStart[®] Afu Uracil-DNA Glycosylase (UDG) incubated for 30 minutes at 25°C releases <3 pmol per minute (<5% activity) of a fluorescently labeled 47-mer single-stranded DNA oligonucleotide containing a single uracil base.

Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in ThermoPol[®] Reaction Buffer containing 1 µg of Lambda DNA and a minimum of 20 units of WarmStart[®] Afu Uracil-DNA Glycosylase (UDG) incubated for 16 hours at 37°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 ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

RNase Activity (Extended Digestion) - A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 2 units of WarmStart[®] 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 fluorescent detection.

Single Stranded DNase Activity (FAM-Labeled Oligo) - 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.

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Quality Approver

