

New England Biolabs Product Specification

Product Name:	<i>Thermostable RNase H</i>
Catalog #:	<i>M0523S</i>
Concentration:	<i>5,000 units/ml</i>
Unit Definition:	<i>One unit is defined as the amount of enzyme required to produce 1 nmol of ribonucleotides from 40 picomoles of a fluorescently labeled 25 base pair RNA-DNA hybrid in a total reaction volume of 50 µl in 20 minutes at 50°C.</i>
Shelf Life:	<i>24 months</i>
Storage Temp:	<i>-20°C</i>
Storage Conditions:	<i>50 mM Tris-HCl, 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1% Triton®X-100, 50% Glycerol (pH 7.5 @ 25°C)</i>
Specification Version:	<i>PS-M0523S v1.0</i>
Effective Date:	<i>29 Jan 2018</i>

Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking) - A 50 µl reaction in RNase H Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 25 units of Thermostable RNase H incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

Exonuclease Activity (Radioactivity Release) - A 50 µl reaction in RNase H Reaction Buffer containing 1 µg of a mixture of single and double-stranded [³H] *E. coli* DNA and a minimum of 25 units of Thermostable RNase H incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

Protein Purity Assay (SDS-PAGE) - Thermostable RNase H is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 5 units of Thermostable RNase H is screened for the presence of *E. coli* genomic DNA using SYBR® Green qPCR with primers specific for the *E. coli* 16S rRNA locus. Results are quantified using a standard curve generated from purified *E. coli* genomic DNA. The measured level of *E. coli* genomic DNA contamination is ≤ 1 *E. coli* genome.

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 5 units of Thermostable RNase H is incubated at 37°C. After incubation for 4 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.



Date 29 Jan 2018

Derek Robinson
Director of Quality Control

