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## New England Biolabs Product Specification

Product Name: RNase H
Catalog #: M0297S/L
Concentration: 5,000 units/ml

Unit Definition: One unit is defined as the amount of enzyme required to produce 1 nmol of ribonucleotides from 20 picomoles of a

fluorescently labeled 50 base pair RNA-DNA hybrid in a total reaction volume of 50 µl in 20 minutes at 37°C.

Shelf Life: 24 months
Storage Temp: -20°C

Storage Conditions: 10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 200 µg/ml rAlbumin, 50 % Glycerol, (pH 7.4 @.

25°C)

Specification Version: PS-M0297S/L v2.0

Effective Date: 16 Jul 2024

## Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking) - A 50  $\mu$ l reaction in RNase H Reaction Buffer containing 1  $\mu$ g of supercoiled PhiX174 DNA and a minimum of 50 units of 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, Single Stranded) - A 50  $\mu$ l reaction in RNase H Reaction Buffer containing 1  $\mu$ g of single stranded [  $^3$ H] *E. coli* DNA and a minimum of 50 units of RNase H incubated for 30 minutes at 37°C releases <0.1 of the total radioactivity.

Protein Purity Assay (SDS-PAGE) - 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 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  $\leq 1$  *E. coli* genome.

RNase Activity (Extended Digestion) - A 10  $\mu$ l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1  $\mu$ l of RNase H 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.









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Date 16 Jul 2024

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