

240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

New England Biolabs Product Specification

Product Name:	Induro® Reverse Transcriptase
Catalog #:	M0681S/L/X
Concentration:	200,000 units/ml
Unit Definition:	One unit is defined as the amount of enzyme that will incorporate 1 nmol of dTTP into acid-insoluble material in a total reaction volume of 50 μ l in 10 minutes at 55°C using poly(rA)•oligo(dT)18 as template.
Shelf Life:	24 months
Storage Temp:	-20°C
Storage Conditions:	20 mM Tris-HCl, 300 mM NaCl, 0.1 mM EDTA, 50% Glycerol, (pH 7.5 @ 25°C)
Specification Version:	PS-M0681S/L/X v2.0
Effective Date:	10 Feb 2023

Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking) - A 50 μ l reaction in NEBuffer 2 containing 1 μ g of supercoiled PhiX174 DNA and a minimum of 200 units of Induro® Reverse Transcriptase incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

Functional Testing (RT-PCR, length) - 200 units of Induro® Reverse Transcriptase is tested for performance in a 20 μ l reaction containing 1X Induro® RT Reaction Buffer and 1 μ g human total RNA. The length of the product is verified by amplification using 1 μ l of the RT reaction and 33 cycles of PCR amplification resulting in the expected 9.3kb product as determined by agarose gel electrophoresis.

Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in NEBuffer 2 containing 1 µg of T3 or T7 DNA in addition to a reaction containing Lambda-HindIII DNA and a minimum of 200 units of Induro® Reverse Transcriptase 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) - Induro[®] Reverse Transcriptase is \geq 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 200 units of Induro® Reverse Transcriptase 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.



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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 200 units of Induro® Reverse Transcriptase 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.

Single Stranded DNase Activity (FAM-Labeled Oligo) - A 50 μ l reaction in 1X CutSmart® Buffer containing a 20 nM solution of a fluorescent internal labeled oligonucleotide and a minimum of 200 units of Induro® Reverse Transcriptase incubated for 16 hours at 37°C yields <10% degradation as determined by capillary electrophoresis.

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit <u>www.neb.com/trademarks</u> for additional information.

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Lauren Brown Quality Approver



Date 10 Feb 2023

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