

## New England Biolabs Certificate of Analysis

**Product Name:** *M-MuLV Reverse Transcriptase Reaction Buffer*  
**Catalog #:** B0253S  
**Concentration:** 10X Concentrate  
**Lot #:** 0121704  
**Assay Date:** 04/2017  
**Expiration Date:** 4/2020  
**Storage Temp:** -20°C  
**Composition (1X):** 50 mM Tris-HCl, 75 mM KCl, 3 mM MgCl<sub>2</sub>, 10 mM DTT, (pH 8.3 @ 25°C)  
**Specification Version:** PS-B0253S v1.0  
**Effective Date:** 28 Apr 2017

Assay Name/Specification (minimum release criteria)	Lot #0121704
<b>Endonuclease Activity (Nicking, Buffer)</b> - A 50 µl reaction in 2X M-MuLV Reverse Transcriptase Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	<b>Pass</b>
<b>Non-Specific DNase Activity (16 hour, Buffer)</b> - A 50 µl reaction in 2X M-MuLV Reverse Transcriptase Reaction Buffer containing 1 µg of T3 DNA in addition to a reaction containing Lambda-HindIII DNA incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	<b>Pass</b>
<b>pH (buffers/solutions)</b> - The pH of 10X M-MuLV Reverse Transcriptase Reaction Buffer is between pH 8.2 and 8.4 at 25°C.	<b>Pass</b>
<b>Phosphatase Activity (pNPP, Buffer)</b> - A 200 µl reaction in 1M Diethanolamine @ pH 9.8 and 0.5 mM MgCl <sub>2</sub> containing 2.5 mM <i>p</i> -Nitrophenyl Phosphate (pNPP) and a minimum of 40 µl M-MuLV Reverse Transcriptase Reaction Buffer incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.	<b>Pass</b>
<b>qPCR DNA Contamination (<i>E. coli</i> Genomic, Buffer)</b> - A minimum of 1 µl of M-MuLV Reverse Transcriptase Reaction Buffer is screened for the presence of <i>E. coli</i> genomic DNA using SYBR® Green qPCR with primers specific for the <i>E. coli</i> 16S rRNA locus. Results are quantified using a standard curve generated from purified <i>E. coli</i> genomic DNA. The measured level of <i>E. coli</i> genomic DNA contamination is ≤ 1 <i>E. coli</i> genome.	<b>Pass</b>



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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 1 µl of M-MuLV Reverse Transcriptase Reaction Buffer 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.	Pass



Authorized by  
Karen Moreira  
28 Apr 2017



Inspected by  
Tony Spear-Alfonso  
20 Sep 2017

