

## New England Biolabs Certificate of Analysis

Product Name: *Template Switching RT Enzyme Mix*  
 Catalog Number: *M0466S*  
 Concentration: *10 X Concentrate*  
 Packaging Lot Number: *10139835*  
 Expiration Date: *02/2023*  
 Storage Temperature: *-20°C*  
 Specification Version: *PS-M0466S/L v1.0*

| Template Switching RT Enzyme Mix Component List |                                  |            |                      |
|---|----------------------------------|------------|----------------------|
| NEB Part Number                                 | Component Description            | Lot Number | Individual QC Result |
| M0466SVIAL                                      | Template Switching RT Enzyme Mix | 10139834   | Pass                 |
| B0466SVIAL                                      | Template Switching RT Buffer     | 10139755   | Pass                 |

| Assay Name/Specification   | Lot # 10139835 |
|--|----------------|
| <p><b>RNase Activity (Extended Digestion)</b><br/>           A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 µl of Template Switching RT Enzyme Mix is incubated at 37°C. After incubation for 16 hours, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</p>  | Pass           |
| <p><b>qPCR DNA Contamination (E. coli Genomic)</b><br/>           A minimum of 1 µl of Template Switching RT Enzyme Mix 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.</p>   | Pass           |
| <p><b>Non-Specific DNase Activity (16 Hour)</b><br/>           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 1 µl of Template Switching RT Enzyme Mix incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.</p>  | Pass           |
| <p><b>Functional Testing (Library Construction, Single Cell RNA)</b><br/>           Template Switching RT Enzyme Mix and Template Switching RT Buffer are functionally validated and compared to previous lots through construction of libraries made from single cells and commercially available RNA using input amounts between 2 pg and 200 ng. Libraries made from previous and current lots are sequenced together on the same</p> | Pass           |

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|---|----------------|
| Illumina® flow cell and compared across various metrics including library yield, individual transcript abundance, 5'-3' transcript coverage, percent ribosomal RNA, and fraction of reads mapping to a reference. |                |

This product has been tested and shown to be in compliance with all specifications.

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15 Mar 2022



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15 Mar 2022