

## New England Biolabs Certificate of Analysis

**Product Name:** *Mismatch Endonuclease I*  
**Catalog Number:** M0678S  
**Concentration:** 80,000 U/ml  
**Unit Definition:** One unit is defined as the amount of enzyme required to cleave  $\geq 50\%$  of 0.2 pmol of a fluorescently labeled 60mer oligonucleotide duplex containing a single T-T mismatch in 30 minutes at 37°C in a total reaction volume of 20  $\mu$ l in 1X NEBuffer r2.1.  
**Packaging Lot Number:** 10115599  
**Expiration Date:** 07/2023  
**Storage Temperature:** -20°C  
**Storage Conditions:** 500 mM NaCl, 10 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, (pH 7.4 @ 25°C)  
**Specification Version:** PS-M0678S v1.0

Mismatch Endonuclease I Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0678SVIAL	Mismatch Endonuclease I	10115601	Pass
B7202SVIAL	NEBuffer™ 2.1	10099548	Pass

Assay Name/Specification	Lot # 10115599
<p><b>Single Stranded DNase Activity (FAM-Labeled Oligo)</b>            A 50 <math>\mu</math>l reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent internal labeled oligonucleotide and a minimum of 5 <math>\mu</math>l of Mismatch Endonuclease I incubated for 16 hours at 37°C yields &lt;5% degradation as determined by capillary electrophoresis.</p>	Pass
<p><b>RNase Activity (Extended Digestion)</b>            A 10 <math>\mu</math>l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 80 units of Mismatch Endonuclease I 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>DNase Activity (Labeled Oligo, 3' extension)</b>            A 50 <math>\mu</math>l reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent labeled double-stranded oligonucleotide containing a 3' extension and a minimum of 5 <math>\mu</math>l of Mismatch Endonuclease I incubated for 16 hours at 37°C yields &lt;5% degradation as determined by capillary electrophoresis.</p>	Pass

Assay Name/Specification	Lot # 10115599
<p><b>Double Stranded DNase Activity (Labeled Oligo)</b> A 50 µl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent labeled double-stranded oligonucleotide containing a blunt end and a minimum of 5 µl of Mismatch Endonuclease I incubated for 16 hours at 37°C yields &lt;5% degradation as determined by capillary electrophoresis.</p>	<b>Pass</b>
<p><b>Non-Specific DNase Activity (16 Hour)</b> A 50 µl reaction in NEBuffer™ r2.1 containing 1 µg of Lambda-HindIII DNA and a minimum of 400 units of Mismatch Endonuclease I incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.</p>	<b>Pass</b>
<p><b>Protein Purity Assay (SDS-PAGE)</b> Mismatch Endonuclease I is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>
<p><b>qPCR DNA Contamination (E. coli Genomic)</b> A minimum of 80 units of Mismatch Endonuclease I 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>	<b>Pass</b>

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

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Packaging Quality Control Inspector  
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