

## New England Biolabs Product Specification

<i>Product Name:</i>	<i>EcoGII Methyltransferase</i>
<i>Catalog #:</i>	<i>M0603S/L</i>
<i>Concentration:</i>	<i>5,000 units/ml</i>
<i>Unit Definition:</i>	<i>One unit is defined as the amount of enzyme required to completely protect 100 ng of a FAM-labeled dsDNA substrate in 30 minutes at 37°C in a total reaction volume of 20 µL against cleavage by MboI restriction endonuclease as determined by capillary electrophoresis.</i>
<i>Shelf Life:</i>	<i>24 months</i>
<i>Storage Temp:</i>	<i>-20°C</i>
<i>Storage Conditions:</i>	<i>250 mM NaCl, 10 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, 0.15 % TritonX-100, 180 µg/ml BSA, (pH 7.4 @ 25°C)</i>
<i>Specification Version:</i>	<i>PS-M0603S/L v2.0</i>
<i>Effective Date:</i>	<i>15 Apr 2024</i>

### Assay Name/Specification (minimum release criteria)

**Endonuclease Activity (Nicking)** - A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of supercoiled pBR322 DNA and a minimum of 25 units of EcoGII Methyltransferase 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)** - A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [<sup>3</sup>H] *E. coli* DNA and a minimum of 25 units of EcoGII Methyltransferase incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

**Functional Testing (Methyltransferase)** - A 20 µl reaction in rCutSmart™ Buffer supplemented with 160 µM SAM containing 100 ng of an 80 bp FAM-labeled dsDNA substrate and 0.5 units of EcoGII Methyltransferase incubated for 30 minutes at 37°C followed by heat inactivation results in ≥ 70% protection from digestion with 5 units of MboI incubated at 37°C for 30 minutes as determined by capillary electrophoresis.

**Non-Specific DNase Activity (16 Hour)** - A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and a minimum of 25 units of EcoGII Methyltransferase 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)** - EcoGII Methyltransferase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.





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Assay Name/Specification (minimum release criteria)
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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 EcoGII Methyltransferase 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.
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Date 15 Apr 2024

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Quality Approver

