New England Biolabs  
Certificate of Analysis

**Product Name:** MfeI  
**Catalog #:**  R0589S/L  
**Concentration:**  10,000 units/ml  
**Unit Definition:** One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA in 1 hour at 37°C in a total reaction volume of 50 µl.  
**Lot #:**  0081409  
**Assay Date:**  09/2014  
**Expiration Date:**  9/2015  
**Storage Temp:**  -20 ºC  
**Storage Conditions:**  50 mM NaCl, 10 mM Tris-Cl (pH 7.4), 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 200 µg/ml BSA  
**Specification Version:**  PS-R0589S/L v1.0  
**Effective Date:**  15 Nov 2013

<table>
<thead>
<tr>
<th>Assay Name/Specification (minimum release criteria)</th>
<th>Lot #0081409</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blue-White Screening (Terminal Integrity)</strong> - A sample of LITMUS28i vector linearized with a 10-fold excess of MfeI, religated and transformed into an E. coli strain expressing the LacZ beta fragment gene results in &lt;1% white colonies.</td>
<td>Pass</td>
</tr>
<tr>
<td><strong>Endonuclease Activity (Nicking)</strong> - A 50 µl reaction in CutSmart™ Buffer containing 1 µg of supercoiled pUC19 DNA and a minimum of 10 units of MfeI incubated for 4 hours at 37°C results in &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</td>
<td>Pass</td>
</tr>
<tr>
<td><strong>Exonuclease Activity (Radioactivity Release)</strong> - A 50 µl reaction in CutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [³H]E. coli DNA and a minimum of 10 units of MfeI incubated for 4 hours at 37°C releases &lt;0.1% of the total radioactivity.</td>
<td>Pass</td>
</tr>
<tr>
<td><strong>Ligation and Recutting (Terminal Integrity)</strong> - After a 20-fold over-digestion of Lambda DNA with MfeI, &gt;95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, &gt;95% can be recut with MfeI.</td>
<td>Pass</td>
</tr>
<tr>
<td><strong>Non-Specific DNase Activity (16 Hour)</strong> - A 50 µl reaction in CutSmart™ Buffer containing 1 µg of Lambda DNA and a minimum of 30 Units of MfeI incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.</td>
<td>Pass</td>
</tr>
</tbody>
</table>
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* The BSA in this product has been granted an EDQM "Certificate of Suitability" from the European Directorate for the Quality of Medicines (# R1-CEP-2003-204-Rev00) and has been granted a USDA Certificate for Export of Bovine Blood Plasma/Serum for Manufacture into Pharmaceutical Products.

M.W. Southworth
Authorized by
Maurice Southworth
15 Nov 2013

Anthony Francis
Inspected by
Anthony Francis
10 Oct 2014