

## New England Biolabs Certificate of Analysis

**Product Name:** *Esp3I*  
**Catalog Number:** *R0734L*  
**Concentration:** *10,000 U/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.*  
**Packaging Lot Number:** *10204863*  
**Expiration Date:** *04/2025*  
**Storage Temperature:** *-20°C*  
**Storage Conditions:** *300 mM NaCl , 10 mM Tris-HCl , 1 mM DTT , 0.1 mM EDTA , 50 % Glycerol , 500 µg/ml BSA, (pH 7.4 @ 25°C)*  
**Specification Version:** *PS-R0734S/L v1.0*

Esp3I Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
R0734LVIAL	Esp3I	10185076	Pass
B7024AVIAL	Gel Loading Dye, Purple (6X)	10198639	Pass
B6004SVIAL	rCutSmart™ Buffer	10198644	Pass

Assay Name/Specification	Lot # 10204863
<b>Exonuclease Activity (Radioactivity Release)</b> A 50 µl reaction in CutSmart® Buffer containing 1 µg of a mixture of single and double-stranded [ <sup>3</sup> H] E. coli DNA and a minimum of 100 units of Esp3I incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
<b>Functional Testing (15 minute Digest)</b> A 50 µl reaction in CutSmart® Buffer containing 1 µg of Lambda DNA and 1 µl of Esp3I incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.	Pass
<b>Ligation and Recutting (Terminal Integrity)</b> After a 10-fold over-digestion of Lambda DNA with Esp3I, ≥95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, ~75% can be recut with Esp3I.	Pass
<b>Non-Specific DNase Activity (16 Hour)</b> A 50 µl reaction in CutSmart® Buffer containing 1 µg of Lambda DNA and a minimum of 100 units of Esp3I incubated for 16 hours at 37°C results in a DNA pattern free of	Pass

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detectable nuclease degradation as determined by agarose gel electrophoresis.	
<p><b>Protein Purity Assay (SDS-PAGE)</b> Esp3I is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>
<p><b>RNase Activity (Extended Digestion)</b> A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 10 units of Esp3I 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>	<b>Pass</b>

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

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