

## New England Biolabs Certificate of Analysis

**Product Name:** Zral  
**Catalog Number:** R0659L  
**Concentration:** 10,000 U/ml  
**Unit Definition:** One unit is defined as the amount of enzyme required to digest 1 µg Lambda DNA in rCutSmart™ Buffer in 1 hour at 37°C in a total reaction volume of 50 µl.  
**Packaging Lot Number:** 10238076  
**Expiration Date:** 03/2026  
**Storage Temperature:** -20°C  
**Storage Conditions:** 10 mM Tris-HCl, 300 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 500 µg/ml rAlbumin (pH 7.4 @ 25°C)  
**Specification Version:** PS-R0659S/L/V v2.0

### Zral Component List

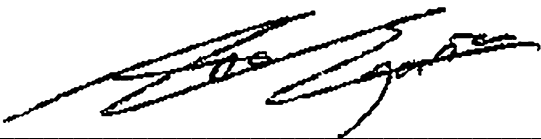
NEB Part Number	Component Description	Lot Number	Individual QC Result
R0659LVIAL	Zral	10230744	Pass
B6004SVIAL	rCutSmart™ Buffer	10233337	Pass

Assay Name/Specification	Lot # 10238076
<b>Endonuclease Activity (Nicking)</b> A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of supercoiled M13mp18 DNA and a minimum of 10 units of Zral incubated for 4 hours at 37°C results in <20% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
<b>Exonuclease Activity (Radioactivity Release)</b> 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 100 units of Zral incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
<b>Ligation and Recutting (Terminal Integrity)</b> After a 10-fold over-digestion of Lambda DNA with Zral, >95% of the DNA fragments can be ligated with T4 DNA ligase in 16 hours at 16°C. Of these ligated fragments, >95% can be recut with Zral.	Pass
<b>Non-Specific DNase Activity (16 Hour)</b> A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and a minimum of 10 units of Zral incubated for 16 hours at 37°C results in a DNA pattern free of	Pass

Assay Name/Specification	Lot # 10238076
detectable nuclease degradation as determined by agarose gel electrophoresis.	
<b>Protein Purity Assay (SDS-PAGE)</b> Zral is $\geq 95\%$ pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	<b>Pass</b>
<b>qPCR DNA Contamination (E. coli Genomic)</b> A minimum of 10 units of Zral 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 $\leq 1$ E. coli genome.	<b>Pass</b>

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

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25 Apr 2024



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