

## New England Biolabs Product Specification

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|-------------------------------|---|
| <b>Product Name:</b>          | <i>AleI-v2</i>  |
| <b>Catalog #:</b>             | <i>R0685S/L</i>   |
| <b>Concentration:</b>         | <i>10,000 units/ml</i>  |
| <b>Unit Definition:</b>       | <i>One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA in rCutSmart Buffer in 1 hour at 37°C in a total reaction volume of 50 µl.</i> |
| <b>Shelf Life:</b>            | <i>12 months</i>  |
| <b>Storage Temp:</b>          | <i>-20°C</i>  |
| <b>Storage Conditions:</b>    | <i>10 mM Tris-HCl, 200 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 200 µg/ml rAlbumin, 50 % Glycerol, (pH 7.4 @ 25°C)</i>   |
| <b>Specification Version:</b> | <i>PS-R0685S/L v2.0</i>   |
| <b>Effective Date:</b>        | <i>03 May 2023</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 30 units of AleI-v2 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 100 units of AleI-v2 incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

**Ligation and Recutting (Terminal Integrity)** - After a 10-fold over-digestion of Lambda DNA with AleI-v2, >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 AleI-v2.

**Non-Specific DNase Activity (16 Hour)** - A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of Lambda DNA and a minimum of 50 units of AleI-v2 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)** - AleI-v2 is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

**qPCR DNA Contamination (*E. coli* Genomic)** - A minimum of 10 units of AleI-v2 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.



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Date 03 May 2023

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Nancy Considine  
Quality Approver

