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# New England Biolabs
# Product Specification

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|---|---|
| *Product Name:* | *KasI* |
| *Catalog #:* | *R0544S/L* |
| *Concentration:* | *5,000 units/ml* |
| *Unit Definition:* | *One unit is defined as the amount of enzyme required to digest 1 µg of pBR322 DNA in rCutSmart Buffer in 1 hour at 37°C in a total reaction volume of 50 µl.* |
| *Shelf Life:* | *12 months* |
| *Storage Temp:* | *-80°C* |
| *Storage Conditions:* | *10 mM Tris-HCl, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 200 µg/ml rAlbumin, 50% Glycerol, (pH 7.4 @ 25°C)* |
| *Specification Version:* | *PS-R0544S/L v4.0* |
| *Effective Date:* | *07 Nov 2023* |

| **Assay Name/Specification (minimum release criteria)** |
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| **Blue-White Screening (Terminal Integrity)** - A sample of LITMUS38i vector linearized with a 10-fold excess of KasI, religated and transformed into an *E. coli* strain expressing the LacZ beta fragment gene results in <1% white colonies. |
| **Exonuclease Activity (Radioactivity Release)** - A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of a mixture of single and double-stranded [ $^{3}$H] *E. coli* DNA and a minimum of 5 units of KasI incubated for 4 hours at 37°C releases <0.1% of the total radioactivity. |
| **Ligation and Recutting (Terminal Integrity)** - After a 20-fold over-digestion of pBR322 DNA with KasI, >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 KasI. |
| **Non-Specific DNase Activity (16 Hour)** - A 50 µl reaction in rCutSmart™ Buffer containing 1 µg of pBR322 DNA and a minimum of 5 units of KasI 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)** - KasI is >95% pure as determined by SDS PAGE analysis using Coomassie Blue detection. |
| **qPCR DNA Contamination (*E. coli* Genomic)** - A minimum of 5 units of KasI 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. |



Nancy Considine
Quality Approver

Date 07 Nov 2023