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New England Biolabs
Product Specification

Product Name:	*T4 RNA Ligase 2, truncated K227Q*
Catalog #:	*M0351S/L*
Concentration:	*200,000 units/ml*
Unit Definition:	*200 units is defined as the amount of enzyme required to give 80% ligation of a 31-mer RNA to the pre-adenylated end of a 17-mer DNA in a total reaction volume of 20 µl in 1 hour at 25°C.*
Shelf Life:	*24 months*
Storage Temp:	*-20°C*
Storage Conditions:	*100 mM NaCl , 10 mM Tris-HCl , 1 mM DTT , 0.1 mM EDTA , 50 % Glycerol, (pH 7.4 @ 25°C)*
Specification Version:	*PS-M0351S/L v2.0*
Effective Date:	*11 Jul 2018*

Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking) - A 50 µl reaction in T4 RNA Ligase Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 200 units of T4 RNA Ligase 2, truncated K227Q 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 T4 RNA Ligase Reaction Buffer containing 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA and a minimum of 200 units of T4 RNA Ligase 2, truncated K227Q incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

Phosphatase Activity (pNPP) - A 200 ul reaction in 1M Diethanolamine, pH 9.8, 0.5 mM $MgCl_2$ containing 2.5 mM *p*-Nitrophenyl Phosphate (pNPP) and a minimum of 200 units of T4 RNA Ligase 2, truncated K227Q incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.

Protein Purity Assay (SDS-PAGE) - T4 RNA Ligase 2, truncated K227Q is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

RNase Activity (Extended Digestion) - A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 200 units of T4 RNA Ligase 2, truncated K227Q is incubated at 37°C. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.

Date 11 Jul 2018

Derek Robinson
Director of Quality Control

ISO 9001 Registered Quality Management
ISO 14001 Registered Environmental Management
ISO 13485 Registered Medical Devices