NEW ENGLAND BioLabs® Inc.

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# New England Biolabs
# Product Specification

| | |
|---|---|
| *Product Name:* | *Hi-T4™ DNA Ligase* |
| *Catalog #:* | *M2622S/L* |
| *Concentration:* | *400,000 units/ml* |
| *Unit Definition:* | *One unit is defined as the amount of enzyme required to give 50% ligation of 6 µg of Lambda-HindIII DNA in 30 minutes at 25°C in a total reaction volume of 20 µl.* |
| *Shelf Life:* | *24 months* |
| *Storage Temp:* | *-20°C* |
| *Storage Conditions:* | *10 mM Tris-HCl , 50 mM KCl , 1 mM DTT , 0.1 mM EDTA , 50 % Glycerol, (pH 7.4 @ 25°C)* |
| *Specification Version:* | *PS-M2622S/L v2.0* |
| *Effective Date:* | *21 Jul 2022* |

**Assay Name/Specification (minimum release criteria)**

**DNase Activity (Labeled Oligo, 3' extension)** - A 50 µl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent labeled double-stranded oligonucleotide containing a 3' extension and a minimum of 2000 units of Hi-T4™ DNA Ligase incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.

**DNase Activity (Labeled Oligo, 5' extension)** - A 50 µl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent labeled double-stranded oligonucleotide containing a 5' extension and a minimum of 2000 units of Hi-T4™ DNA Ligase incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.

**Double Stranded DNase Activity (Labeled Oligo)** - A 50 µl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent labeled double-stranded oligonucleotide containing a blunt end and a minimum of 2000 units of Hi-T4™ DNA Ligase incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis.

**Endonuclease Activity (Nicking)** - A 50 µl reaction in NEBuffer 1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 400 units of Hi-T4™ DNA Ligase incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

**Non-Specific DNase Activity (16 Hour)** - A 50 µl reaction in NEBuffer 1 containing 1 µg of CIP-treated Lambda-HindIII DNA and a minimum of 400 units of Hi-T4™ DNA Ligase incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

**Protein Concentration (A280)** - The concentration of Hi-T4™ DNA Ligase greater than or equal to 0.4 mg/ml as determined by UV absorption at 280 nm. Protein concentration is determined by the Pace method using the extinction coefficient of 57,675 and molecular weight of 56,806 daltons for Hi-T4™ DNA Ligase (Pace, C.N. et al. (1995) Protein Sci., 4, 2411-2423).

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| Assay Name/Specification (minimum release criteria) |
|---|
| **Protein Purity Assay (SDS-PAGE)** - Hi-T4™ DNA Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection. |
| **qPCR DNA Contamination (*E. coli* Genomic)** - A minimum of 400 units of Hi-T4™ DNA Ligase 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. |
| **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 1 µl of Hi-T4™ DNA Ligase 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. |
| **Single Stranded DNase Activity (FAM-Labeled Oligo)** - A 50 µl reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent internal labeled oligonucleotide and a minimum of 2000 units of Hi-T4™ DNA Ligase incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis. |



Date 21 Jul 2022

Derek Robinson
Director, Quality Control