NEW ENGLAND BioLabs Inc.

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# New England Biolabs
# Certificate of Analysis

| | |
|---|---|
| *Product Name:* | *T4 Gene 32 Protein* |
| *Catalog #:* | *M0300S/L* |
| *Concentration:* | *10 mg/ml* |
| *Lot #:* | *0241804* |
| *Assay Date:* | *04/2018* |
| *Expiration Date:* | *04/2020* |
| *Storage Temp:* | *-20°C* |
| *Storage Conditions:* | *20 mM Tris-HCl, 100 mM NaCl, 0.5 mM DTT, 1 mM EDTA, 50% Glycerol, (pH 8.0 @ 25°C)* |
| *Specification Version:* | *PS-M0300S/L v1.0* |
| *Effective Date:* | *08 Feb 2018* |

| **Assay Name/Specification** (minimum release criteria) | **Lot #0241804** |
|---|---|
| **Endonuclease Activity (Nicking) -** A 50 µl reaction in NEBuffer 4 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 10 µg of T4 Gene 32 Protein incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis. | **Pass** |
| **Exonuclease Activity (Radioactivity Release) -** A 50 µl reaction in NEBuffer 4 containing 1 µg of a mixture of single and double-stranded [ $^{3}$H] *E. coli* DNA and a minimum of 10 µg of T4 Gene 32 Protein incubated for 4 hours at 37°C releases <0.1% of the total radioactivity. | **Pass** |
| **Functional Testing (Single Stranded DNA Binding - FAM Labeled Oligo) -** A 20 µl reaction in NEBuffer 4 containing 20 µM FAM-labeled 50-mer and a maximum of 80 µg of T4 Gene 32 Protein incubated for 30 minutes at 37°C produces a mobility shift in >95% of the starting material as determined by TBE gel electrophoresis and UV imaging. | **Pass** |
| **Non-Specific DNase Activity (16 Hour) -** A 50 µl reaction in NEBuffer 4 containing 1 µg of Lambda-HindIII DNA and a minimum of 30 µg of T4 Gene 32 Protein incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis. | **Pass** |
| **Phosphatase activity (FAM Labeled Oligo) -** A 50 ul reaction in CutSmart® Buffer containing a 20 nM solution of a fluorescent internal labeled oligonucleotide with a 5' phosphate and a minimum of 10 µg of T4 Gene 32 Protein incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis. | **Pass** |
| **Protein Concentration (A280) -** The concentration of T4 Gene 32 Protein is 10 mg/ml +/- 10% as determined by UV absorption at 280 nm. Protein concentration is determined by the Pace method using the extinction coefficient of 39,670 and molecular weight of 33,506 daltons for T4 Gene 32 Protein (Pace, C.N. et al. (1995) Protein Sci., 4, 2411-2423). | **Pass** |

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| **Assay Name/Specification** (minimum release criteria) | **Lot #0241804** |
|---|---|
| **Protein Purity Assay (SDS-PAGE)** - T4 Gene 32 Protein is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection. | **Pass** |
| **qPCR DNA Contamination (E. coli Genomic)** - A minimum of 10 µg of T4 Gene 32 Protein 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. | **Pass** |
| **RNase Activity Assay (2 Hour Digestion)** - A 10 µl reaction in NEBuffer 4 containing 40 ng of fluorescein labeled RNA transcript and a minimum of 10 µg of T4 Gene 32 Protein incubated for 2 hours at 37°C results in no detectable degradation of the RNA as determined by gel electrophoresis using fluorescent detection. | **Pass** |
| **RNase Activity (Extended Digestion)** - A 10 µl reaction in NEBuffer 4 containing 40 ng of fluorescein labeled RNA transcript and a minimum of 10 µg of T4 Gene 32 Protein 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. | **Pass** |
| **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 10 µg of T4 Gene 32 Protein incubated for 16 hours at 37°C yields <5% degradation as determined by capillary electrophoresis. | **Pass** |

Authorized by
Derek Robinson
08 Feb 2018

Inspected by
Bo Wu
06 Apr 2018

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