T4 Gene 32 Protein

New Quality Control Standards

M0300S

Description: T4 Gene 32 Protein is a single-stranded DNA (ssDNA) binding protein required for bacteriophage T4 replication and repair (1-2). It cooperatively binds to and stabilizes transiently formed regions of ssDNA and plays an important structural role during T4 phage replication (3). It has also been used extensively to stabilize and mark regions of ssDNA for electron microscopic examination of intracellular DNA structures (4-5). Recently, it has been shown to improve restriction enzyme digestion (6), improve the yield and efficiency of reverse transcription (RT) reactions during RT-PCR (7-9), enhance T4 DNA polymerase activity (10-11), as well as increase the yield of PCR products (12).

Source: An E. coli strain carrying a plasmid that overexpresses the gene 32 protein of T4 phage

Applications:

- Stabilization and marking of ssDNA structures (4-5)
- Increase yield and specificity of PCR products from soil samples (10-12)
- Increase yield and processivity of RT during RT-PCR (7-9)

Reagents Supplied with Enzyme:

10X NEBuffer 4, 100 µg

Reaction Conditions: 1X NEBuffer 4, Incubate at 37°C.

1X NEBuffer 4:

- 50 mM potassium acetate
- 20 mM Tris-acetate
- 10 mM magnesium acetate
- 1 mM DTT
- pH 7.9 @ 25°C

Molecular Weight: 33,506 daltons.

Heat Inactivation: 65°C for 20 minutes.

Protein Concentration (OD 280): The concentration of T4 Gene 32 Protein is 10 mg/ml +/- 5% as determined by UV absorption at 280 nm. Protein concentration is determined by the Pace method using 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).

Quality Control Assays

Single Stranded DNA Binding (FAM labeled oligo):

- A 20 µl reaction in NEBuffer 4 containing 20 µM FAM labeled 21 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 10% TBE gel electrophoresis and uv imaging.

Triple Stranded DNA Binding (FAM labeled oligo):

- A 50 µl reaction in NEBuffer 4 containing 20 µM FAM labeled 21 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 10% TBE gel electrophoresis and uv imaging.

Protein Purification (SDS-PAGE): T4 Gene 32 Protein is >99% pure as determined by SDS PAGE analysis using Coomassie blue detection.

Non-specific DNase Activity (16-hour):

- A 50 µl reaction in NEBuffer 4 containing 1 µg of Lambda DNA (HindIII digest) 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.

Endonuclease Activity (Nicking):

- A 50 µl reaction in NEBuffer 4 containing 1 µg of supercoiled pX174 RF I DNA 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.

Exonuclease Activity (Radioactivity Release):

- A 50 µl reaction in NEBuffer 4 containing 1 µg of a mixture of single and double-stranded [3H] 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.

Heat Inactivation:

Incubate at 37°C.

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(see other side)

CERTIFICATE OF ANALYSIS
Single Stranded Deoxyribonuclease Activity (FAM Labeled Oligo): A 50 µl reaction in NEBuffer 4 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 < 1% degradation as determined by capillary electrophoresis.

Phosphatase Activity (FAM Labeled Oligo): A 50 µl reaction in NEBuffer 4 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 < 1% degradation as determined by capillary electrophoresis.

RNase Activity (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 fluorescence detection.

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 Syber 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 less than 1 E. coli genome.

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 fluorescence detection.

References: