Product Information	
T4 Gene 32 Protein	
Part Number	Y9130L
Concentration	10 mg/mL
Unit Size	1.0 mg
Storage Temperature	-25ºC to -15ºC
Lot Number	
Reference Number	

Product Description: T4 Gene 32 Protein is a single-stranded DNA binding protein which is required for T4 DNA replication, recombination and repair (1, 2). The protein has exhibited an ability to enhance the performance of several DNA synthesis-related activities, including DNA sequencing in secondary-structure rich regions and PCR amplification (3-6). T4 Gene 32 also greatly stimulates the rate of synthesis of T4 DNA Polymerase on primed-single-stranded substrates (7-8).

Product Specifications						
Y9130						
Assay	**SDS Purity**	**DNA Binding**	**SS Exonuclease**	**DS Exonuclease**	**DS Endonuclease**	***E. coli* DNA Contamination**
Units Tested	**n/a**	**n/a**	**100µg**	**100µg**	**100µg**	**25µg**
Specification	>99%	Functional	<1.0% Released	<1.0% Released	No Conversion	<10 copies

Source of Protein: Purified from a strain of *E. coli* that overexpresses the gene 32 protein from bacteriophage T4.

Molecular weight: 33,506 Daltons

Quality Control Analysis:

DNA Binding of single stranded DNA was measured using a gel shift assay with a single-stranded, fluorescently labeled oligonucleotide. Serial dilutions of the enzyme were made in 1X T4 GP32 reaction buffer(50mM Potassium Acetate, 20mM Tris Acetate, 10mM Magnesium Acetate, 1mM DTT pH 7.9) and added to 10 µL reactions containing a 5'-FAM labeled oligonucleotide substrate, and 1X T4 GP32 Reaction Buffer. Reactions were incubated 20 minutes at 37°C, plunged on ice, and run out on a 15% TBE-Urea gel. DNA binding ability is observed as a band shift in the apparent molecular weight of the oligonucleotide on the TBE-Urea gel.

Protein Concentration (OD_{280}) is determined by OD_{280} absorbance.

Physical Purity is evaluated by SDS-PAGE of concentrated and diluted enzyme solutions followed by silver stain detection. Purity is assessed by comparing the aggregate mass of contaminant bands in the concentrated sample to the mass of the protein of interest band in the diluted sample.

Single-Stranded Exonuclease is determined in a 50 µL reaction containing a radiolabeled single-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.

Double-Stranded Exonuclease is determined in a 50 µl reaction containing a radiolabeled double-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.

Double-Stranded Endonuclease is determined in a 50 µL reaction containing 0.5 µg of plasmid DNA and 10 µL of enzyme solution incubated for 4 hours at 37°C.

***E.coli* 16S rDNA Contamination** is evaluated using 5 µL **r**eplicate samples of enzyme solution denatured and screened in a TaqMan qPCR assay for the presence of contaminating *E.coli* genomic DNA using oligonucleotide primers corresponding to the 16S rRNA locus.

Supplied in: 20 mM Tris-HCl, 100mM NaCl, 0.5mM DTT, 1mM EDTA, 50% glycerol (pH 8.0 at 25ºC).
Usage Instructions: The T4 gene 32 protein is a single-stranded DNA binding protein (SSB) which can be used to increase the performance in PCR or NASBA reactions and for stabilization of single-stranded regions of DNA or RNA (9, 10).

The protein was also be used in combination with T4 DNA Polymerase and T4 DNA Ligase for site-specific mutagenesis application (11).

As a starting point in SSB mediated PCR or NASBA reactions, add T4 gene 32 protein at a concentration range between 20–320 ng/µL per reaction respectively to identify the optimal concentration.

References:

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