Product Information	
TaqIT	
Part Number	P7620L
Concentration	50,000 U/mL
Unit Size	5,000 U
Storage Temperature	-25°C to -15°C
Lot Number	
Reference Number	

Product Description: TaqIT is an exonuclease deficient derivative of Taq DNA polymerase. TaqIT lacks the first 280 amino acids of native Taq polymerase that contain the 5'-3' exonuclease domain. This deletion makes TaqIT slightly more thermostable and has slightly greater fidelity than full length Taq. Like Taq polymerase, TaqIT has no inherent 3'-5' exonuclease activity (1, 2).

Product Specifications						
P7620						
Assay	**SDS Purity**	**Specific Activity**	**SS Exonuclease**	**DS Exonuclease**	**DS Endonuclease**	***E. coli* DNA Contamination**
Units Tested	**n/a**	**n/a**	**500**	**500**	**500**	**500**
Specification	>99%	42,000 U/mg	<1.0% Released	<1.0% Released	No Conversion	<10 copies

Source of Protein: A recombinant *E. coli* strain carrying the TaqIT gene from the thermophilic organism *Thermus Aquaticus*.

Unit Definition: 1 unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid-insoluble material in 30 minutes at 75°C.

Molecular weight: 62.4 KDa

Quality Control Analysis:

Unit Activity is measured using a 2-fold serial dilution method. Dilutions of enzyme were made in a reduced-glycerol (5%) containing Taq-B DNA Polymerase storage solution and added to 50 µL reactions containing Calf Thymus DNA, 25 mM TAPS (pH 9.3), 50 mM KCl, 1 mM DTT, ^{3}H-dTTP and 100 µM dNTPs. Reactions were incubated 10 minutes at 75°C, plunged on ice, and analyzed using the method of Sambrook and Russell (3).

Protein Concentration 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, 222 mM $(NH_4)_2SO_4$, 10 mM 2-mercaptoethanol, 0.1 mM EDTA, 0.1% Brij-58, 50% glycerol (pH 8.6 at 25°C)

Supplied with:
10X TaqIT Reaction Buffer (B7620): 500 mM Tris-HCl, 160 mM $(NH_4)_2SO_4$, 35 mM $MgCl_2$, 0.25% Brij-58 (pH 9.2 at 25°C)

Usage Instructions:
Set up the following reaction mixture in a total volume of 50 µL*

Component	Volume (µL)	Final Concentration
Nuclease free H_2O	x	N/A
10X TaqIT Reaction Buffer (B7620)	5	1X
10 mM dNTP mix	1	200 µM each
Primer 1	x	0.2 µM
Primer 2	x	0.2 µM
DNA template	x	< 1000 ng
TaqIT (P7620)	0.1-0.5	5-25 U

* Volumes can be adjusted as needed.

Cycling conditions**

Step	Temperature	Time	Cycles
Initial Denaturation	94°C	30 sec -3 min	1
Denaturation	94°C	30 sec	25 - 40
Annealing	50-65°C	30 sec	
Extension	72°C	60 sec/kb	
Final Extension	72°C	5 min	1
	4°C	hold	

** Cycling conditions may need to be optimized, depending on the amplicon of interest.

References:
1. Barnes, W.M. (1992) Gene, 112(1):29-35.
2. Karantzeni, I. et al. (2003) Biochem J., 374(Pt 3):785-92.
3. Sambrook, J. and Russell, D.W. (2001) Cold Spring Harbor Laboratory Press, Molecular Cloning: A Laboratory Manual., v3, A8.25-A8.26.

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