WizPure™ Taq DNA Polymerase

- W1301E **500 U** -
- W1301E-5 **2,500 U** -
- W1301 **500 U** dNTP
- W1301-5 **2,500 U** dNTP

Description
WizPure™ Taq DNA Polymerase is a thermally stable, processive, 5′→ 3′ DNA polymerase. The 94 kDa protein possesses an inherent 5′→3′ nick-translation moiety and lacks a 3′→5′ proofreading function.

Kit Contents

Contents	W1301E	W1301E-5	W1301	W1301-5
Taq DNA Polymerase (5U/µl)	100 µl	500 µl	100 µl	500 µl
10X PCR Buffer	2 ml	10 ml	2 ml	10 ml
dNTP mix (2.5 mM each)			1 ml	5 ml

Storage Buffer
20mM Tris-HCl, 1mM dithiothreitol, 0.1mM EDTA, 100mM NaCl, Stabilizer, 50% glycerol, pH 7.5 (25°C).

10X PCR Buffer
100mM Tris-HCl, 500mM KCl, 15mM MgCl2, pH 8.5 (25°C).

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.

Storage Conditions
Store all components at -20ºC in a non-frost-free freezer.

Recommended Protocol
On ice, prepare each of following master mixes, combine, and place in heated (to 95°C) thermal cycler:

For 50µl PCR Reaction	Volume	Final Conc.
Taq DNA Polymerase (5U/µl)	0.2 ~ 1 µl	1 ~ 5 U
10X PCR Buffer	5 µl	1 X
dNTP mix (2.5 mM each)	4 µl	200 µM each
Template	< 500 ng	< 500 ng
Forward Primer	5 ~ 50 pmol	0.1 ~ 1 µM
Reverse Primer	5 ~ 50 pmol	0.1 ~ 1 µM
Distilled water	up to 50 µl	

General Cycling Conditions :

Step	Temp (°C)	Time	Cycle
Initial Denaturation	95	5 min.	1
Denature	95	10 ~ 30 sec.	25 ~ 40
Anneal	50~65	10 ~ 30 sec.	
Extend	72	10 ~ 60 sec.	
Final Extension	72	5 min.	1

Notice:
Cycling conditions may need to be optimized, depending on different primer and template combinations. For example, raise the annealing temperature to prevent non-specific primer binding, increase extension time to generate longer PCR products.

Quality Control Analysis:

Unit Characterization Assay
Specific activity was measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 1X reaction buffer. Reactions were incubated 10 minutes at 75°C, plunged on ice, and analyzed using the method of Sambrook and Russell.

Protein Concentration (OD280) Measurement
A 2.0 µl sample of enzyme was analyzed at OD280 using a spectrophotometer standardized using a 2.0 mg/ml BSA sample and blanked with product storage solution. The observed average measurement of 3 replicate samples was converted to mg/mL using an extinction coefficient of 110,380 and molecular weight of 93,909 Daltons. Acceptance for this assay is +/- 5% of reference sample.

SDS-Page (Physical Purity Assessment)
2.0 µl of concentrated enzyme solution was loaded on a denaturing 4-20% Tris-Glycine SDS-PAGE gel flanked by a broad-range MW marker and 2.0 µl of a 1:100 dilution of the sample. Following electrophoresis, the gel was stained and the samples compared to determine physical purity. The acceptance criteria for this test requires that the aggregate mass of contaminant bands in the concentrated sample do not exceed the mass of the protein of interest band in the dilute sample, confirming greater than 99% purity of the concentrated sample.

Nuclease Contamination Tests:

Single-Stranded Exonuclease Activity
A 50 µl reaction containing 11,000 cpm of a radiolabeled single-stranded DNA substrate and 10 µl of enzyme solution incubated for 4 hours at 37°C resulted in less than 5.0% release of TCA-soluble counts.

Double-Stranded Exonuclease Activity
A 50 µl reaction containing 5,000 cpm of a radiolabeled double-stranded DNA substrate and 10 µl of enzyme solution incubated for 4 hours at 37ºC resulted in less than 0.5% release of TCA-soluble counts.

Endonuclease Activity
A 50 µl reaction containing 0.5 µg of pBR322 DNA and 10 µL of enzyme solution incubated for 4 hours at 37°C resulted in no visually discernible conversion to nicked circular DNA as determined by agarose gel electrophoresis.

E.coli 16S rDNA Contamination Test
Replicate 5 µl samples of enzyme solution were 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. The acceptance criterion for the test is the threshold cycle count (Ct) produced by the average of 3 replicate no template control samples. Based on the correlation between the no template control Ct values, and standard curve data, the detection limit of this assay is <10 copies genome/sample.

Quality Authorized by : Jamie Ahn

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