A.B.T.™ HS-Taq DNA Polymerase

Cat#	Size
• E02-01-50	200 µl (500 U) (without dNTPs)
• E02-02-50	200 µl (500 U) (with dNTPs)

Description: A.B.T.™ HS-Taq DNA Polymerase is hot start Taq DNA polymerase which is an antibody mediated enzyme and highly convenient for real-time PCR, high-specific hot-start PCR and multiplex PCR. The enzyme is an exceptionally processive 5'→3' DNA polymerase but lacks the 3'→5' proofreading and 3'→5' exonuclease activity features.

Kit Components:

Components	E02-01-50	E02-02-50
A.B.T.™ HS-Taq DNA Polymerase (2.5 U/µl)	200 µl	200 µl
10X PCR Buffer	750 µl	750 µl
dNTP Mix (2.5 mM each)	-	600 µl

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

For 50 µl PCR Reaction	Volume	Final Conc.
A.B.T.™ HS-Taq DNA Polymerase (2.5 U/µl)	0.4 - 2 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:

PCR Step	Temp (°C)	Time	Cycle
Initial Denaturation	95	300 sec.	1
Denature	95	10 - 30 sec.	25 - 40
Anneal	50 - 65	10 - 30 sec.	
Extend	72	10 - 60 sec.	
Final Extension	72	300 sec.	1

Notice: Depending on different primer and template combinations cycling conditions can be optimized. For example, raise the annealing temperature to prevent non-specific primer binding. On the other hand, increase extension time to generate longer PCR products.

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

10X PCR Buffer: 500 mM KCl, 100 mM Tris-HCl, 15 mM $MgCl_2$, pH 8.5 (25°C).

Unit Description: 1 unit is defined as the amount of enzyme that will incorporate 10 nmol of deoxyribonucleotides into acid-insoluble material (30 min., 75°C).

Storage Conditions: Store all contents at -20ºC in a freezer.

Quality Control Analysis:
Unit Characterization Assay: Using a 2-fold serial dilution method specific enzyme activity was calculated. 1X Reaction buffer was used as the diluent. Reactions carried out (10 min., 75°C) then placed on ice, and analyzed (Sambrook and Russell method).

Protein Concentration Measurement: Enzyme (2 µl) was analyzed at OD280 using standardized BSA samples, blank and spectrophotometer. Average results of samples (three replicates) were determined. The results were converted to mg/mL using molecular weight (94 kDa) and extinction coefficient value (110,310). Acceptance criteria for this test is ± 5% of reference sample.

Physical Purity Assessment: A certain amount of concentrated enzyme (2.0 µl), 1:100 dilution of the sample and broad range protein molecular weight markers was loaded on gel (4-20% Tris-Glycine SDS-PAGE). The gel was stained by electrophoresis carried out. It is the acceptance criteria that the stained band of the concentrated enzyme does not exceed the stained band of the diluted enzyme. This confirms that the concentrated sample is greater than 99% purity.

Nuclease Contamination Tests:
Single-Stranded Exonuclease Activity: Enzyme (10 µl) and a radiolabeled single-stranded DNA substrate (11,000 cpm) incubated at 37°C (Total volume 50 µl, 4 hours). Release of TCA-soluble counts is less than 5.0%.

Double-Stranded Exonuclease Activity: Enzyme (10 µl) and a radiolabeled double-stranded DNA substrate (5,000 cpm) incubated at 37°C (Total volume 50 µl, 4 hours). Release of TCA-soluble counts is less than 0.5%.

Endonuclease Activity: Enzyme (10 µl) and pBR322 DNA (0.5 µg) substrate incubated at 37°C (Total volume 50 µl, 4 hours). Agarose gel electrophoresis was carried out. Visually noticeable nicked circular DNA was not observed.

E.coli 16S rDNA Contamination Test: To determine the presence of E.coli contamination, replicate 5 µl enzyme samples was denatured. TaqMan qPCR assay was performed using E.coli 16S rRNA locus specific oligonucleotide primers. Acceptance criterion is the threshold cycle values (Ct) values calculated with the average of 3 replicate no template control samples for this test. Considering the correlation between the standard curve data and the Ct values, the detection limit of this test is <10 copies genome / sample.

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