Product information

Product Name: *Taq* DNA Polymerase

With 10X reaction buffer containing 25 mM $MgCl_2$

Catalog No: VN160E, VN161E

Packing Size: 125 µl (1000~2000 x 25 µl rxns) and 50 µl (500~1000 x 25 µl rxns)

Shipping Condition: Ambient temperature

Storage Condition: -20^{0}C

Thermo Stability: Retains at least 85% activity after 1 hour at 95^{0}C

Shelf life: At least 3 years from date of receipt under proper storage conditions

Product Description:

***Taq* DNA Polymerase** is a highly thermostable DNA polymerase from the thermophilic bacterium *Thermus aquaticus*. The enzyme catalyzes 5'→3' synthesis of DNA, has no detectable 3'→5' exonuclease (proofreading) activity and possesses low 5'→3' exonuclease activity. In addition, *Taq* DNA Polymerase exhibits deoxynucleotidyl transferase activity, which frequently results in the addition of extra adenines at the 3'-end of PCR products. Recombinant *Taq* DNA Polymerase is the ideal tool for standard PCR of purified DNA template. This enzyme can be used in real-time PCR both with DNA binding dyes, such as SYBR Green and Eva Green, and TaqMan assay that requires 5'-exonuclease activity.

Source:

Purified from E. coli. K12 carrying a recombinant plasmid.

10X Reaction Buffer:

500 mM Tris-HCl pH 9.1, 160 mM ammonium sulfate, 0.25% Brij-58, and 25 mM magnesium chloride.

Storage Buffer (not provided):

50% Glycerol (v/v), 20 mM Tris-HCl (pH 8.5), 222 mM $(NH4)_2SO4$, 0.1 mM EDTA, 10 mM β-mercaptoethanol and 0.1% Brij-58.

Quality Control:

Enzyme Purification Test

The final preparation of the enzyme shows a 94 kDa single-band (appr. 95% homogeneity) in SDS gel stained with Coomassie Blue.

PCR Amplification

Enzyme titration: A dilution series of 1/2, 1/4, 1/8, 1/16 and 1/32 µl of *Taq* per 50 µl of reaction volume is used to amplify a 2 kb fragment from 1 ng Lambda DNA in 28 cycles. Observed amplification by 1/8 µl enzyme passes the test. In parallel test the PCR performance of the enzyme also matches that of a previous enzyme lot.

VitaNavi Technology LLC. 613 Big Bend Rd, Unit 941, Ballwin MO 63021. Toll free (US and Canada): (800) 586-6927; Phone: (314) 499-8280. Fax: (866) 302-5897. Email: support@vitanavitech.com. Website: https://www.vitanavitech.com.

DNA Contamination Test

Primers specific to a 346 bp span of R coli 16S rRNA gene are employed in a PCR amplification, with no actual E. coli DNA added as template. Defined amounts of E. coli X7029 DNA are used as positive control. After 35 cycles no PCR products are detected except in the positive controls.

Endonuclease

Four amounts of the enzyme, 1.0, 0.5, 0.25 µl and 0.125 µl are incubated with 500 ng supercoiled phiX174 RF DNA, in 50 µl reaction volumes with 1X buffer, for 4 hours at 37°C. No nicking is observed when the enzyme is ≤ 0.5 µl / 50 µl compared to no-enzyme control.

Protocol:

PCR setting for a 25 µl reaction

Reagent	Volume	Final Concentration
10x Taq Mutant Buffer	2.5 µl	1x
dNTP Mix (10 mM)	0.5-1.0 µl	200-400 µM each
Left Primer	Variable	0.2-0.4 µM
Right Primer	Variable	0.2-0.4 µM
DNA template†	Variable	0.1-100 ng
PCR Enhancer Cocktail (optional)*	Titration	Variable
Taq Polymerase**	0.05-0.25 µl	
De-ionized Distilled H_2O	Adjust final volume to 25 µl	-

†DNA amount depends mostly on genome size and target gene copy number.

* For optimal performance, we recommend using one of our PCR Enhancer Cocktails if your samples contain some PCR inhibitors. The PEC is not included with the enzyme and you should order separately if you need it. A titration of PEC is also recommended. Please see the datasheet of PEC for the details.

** To determine specific optimal enzyme concentration, we strongly recommend an enzyme titration test for each target. A good starting amount of enzyme per 25 µl reaction is 0.05 µl for purified DNA. Targets larger than 1 kb may require more enzyme or use LA version.

Typical Cycling Parameters

Initial denaturation	95^0C	2 min
25-40 cycles		
Denaturation	94^0C	10 to 30 sec
Annealing	50^0C to 68^0C	20 to 60 sec
Extension	70^0C	1-2 min / 1kb target
Final Extension	70^0C	5 min
Hold	4^0C	

Troubleshooting guide

No PCR products	Check your PCR setting to see if you miss some components in the PCR master mix.
The bands in agarose gel are smear	Enzyme titration test to find optimal enzyme concentration for your target. Two much enzyme may inhibit the PCR, especially when the target gene is short and easy. Check to see if the DNA or primers degraded.
Low yield of product	Increase PCR cycles. Try gradient annealing temperature. Enzyme titration. Use PCR enhancer cocktail. Redesign primers.
Non-specific products are observed	Try gradient annealing temperature to find optimal annealing temperature for your target. Check GC content of the target. If it is more than 65%, you may need to use PEC-1-GC in the PCR. Check your primers or redesign them if necessary. Use hot-start Taq mutant enzymes.

Note: This product is for R&D use only

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