



Product information

Product Name: **OmniTaq™ LA DNA Polymerase**

With 10X reaction buffer containing 25 mM MgCl₂

Catalog No: VN130E, VN131E, VN132E

Packing Size: 125 µl (500~1000 x 25 µl rxns), 50 µl (200~400 x 25 µl rxns) and 25 µl (100~200 x 25 µl rxns)

Shipping Condition: Ambient temperature

Storage Condition: -20°C

Thermo Stability: Retains at least 85% activity after 1 hour at 95°C

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

Product Description:

OmniTaq™ LA DNA Polymerase is a mutant of Taq polymerase that makes the enzyme resistant to the inhibitory effects of blood, soil and more. It typically remains functional in 20-25% whole blood, especially in the presence of one of our PCR enhancer cocktails, and in some concentrations of crude soil extracts where other commercial enzymes fail. The Long-and-Accurate feature allows for amplification of longer products with higher fidelity and accuracy. This enzyme can be used in real-time PCR with DNA binding dyes, such as SYBR Green and Eva Green, however, it is better to choose Non-LA version in real-time PCR TaqMan assay.

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 - 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 OmniTaq LA 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.

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

Protocol:

PCR setting for a 25 μ l reaction

Reagent	Volume	Final Concentration
10x Taq Mutant Reaction Buffer	2.5 μ l	1x
dNTP Mix (10 mM)	0.5-1 μ l	200-400 μ M each
Left Primer	Variable	0.2-0.4 μ M
Right Primer	Variable	0.2-0.4 μ M
DNA template [†] / Blood / Serum / Plasma	Variable	0.1-100 ng <5 μ l
PCR Enhancer Cocktail (recommended for crude samples)*	Titration	Variable
OmniTaq LA Polymerase**	0.1-0.25 μ l	
De-ionized Distilled H ₂ O	Adjust final volume to 25 μ l	-

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

* For optimal performance, we recommend using one of our PCR Enhancer Cocktails (see PCR enhancers for the detail) that are specially formulated for use with whole blood, serum, plasma or other crude samples. The PEC is not included with the enzyme and you should order separately if you need it. A titration of PEC is recommended to find an optimal amount for your target. 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 μ l reaction is 0.05 μ l for purified DNA templates and 0.125 μ l for crude samples containing 5% or more whole blood, plasma or serum. Targets larger than 1 kb may require more enzyme.

Typical Cycling Parameters

Initial denaturation	95°C	2-5 min (for purified DNA) 6-10 min (for crude sample)
25-40 cycles		
Denaturation	94°C	20 to 40 sec
Annealing	50°C to 68°C	20 to 60 sec
Extension	70°C	1-2 min / 1kb target
Final Extension	70°C	5 min
Hold	4°C	

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. Too 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