For research use only

ISO9001

rTaq Plus DNA Polymerase

Product Description	Quantity	Cat. No.	Remarks
rTaq Plus DNA Polymerase	250 unit	EBT-1317	5 unit/μl
	500 unit	EBT-1318	5 unit/μl

Description

rTaq Plus DNA polymerase is a thermostable DNA polymerase that designed for a reliable amplification of long and complex targets with a robust yield in an extremely short extension time. Due to its high elongation rate (about 100 bp/sec) and high processivity (about 250 bases), long sized products can be generated within relatively shorter running time compared to any other commercially available enzymes (5 kbp within 60 sec extension).

Recombinant rTaq Plus DNA Polymerase is purified from a modified recombinant *E.coli* strain. The enzyme catalyzes the incorporation of nucleotides into duplex DNA in the 5′→3′ direction in the presence of Mg^{2+} at 70-80°C. rTaq Plus DNA Polymerase has no detectable exonuclease activity (5′→3′or 3′→5′) and generates a 3′-dA overhang, suitable for a TA cloning of PCR products.

rTaq Plus DNA polymerase is free from any nucleic acid which can be contaminated from expression host during purification process. That provides robust conditions for amplification of gram-negative bacterial genes, and profiling of these.

rTaq Plus DNA polymerase is provided with 10x optimized reaction buffer.

Storage Buffer

5 unit/μl in 50 mM Tris-HCl, pH8.0, 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.1% NP-40, 0.1% Tween-20, 50% Glycerol.

Unit Definition

One unit of enzyme catalyzes the incorporation of 10 nanomoles of deoxynucleotides into a polynucleotide fraction in 30 min at 72°C.

10x Reaction Buffer

500 mM Tris-HCl, pH7.9, 100 mM KCl, 60 mM $(NH_4)_2SO_4$, 15 mM $MgCl_2$, 1% Triton X-100, 0.1 mg/ml BSA.

QC tests

Activity, SDS-PAGE purity, performance tests, genomic DNA contamination test, confirmation test for the absence of endo and exonucleases.

Storage Condition

Store at -20°C.

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Standard Protocol

1. Prepare 50 μl PCR solution as follows:

PCR grade distilled water :	- μl
10x rTaq Plus buffer :	5 μl
10 mM dNTP mix (2.5 mM each) :	4 μl
Primer (10 pmol/μl) :	1 μl each
Template :	0.1-10 ng
rTaq Plus DNA Polymerase :	0.2-0.5 μl (1-2.5 unit)

Adjust final vol. to 50 μl with PCR grade distilled water

*Note : Making a mixture on ice may reduce a non-specific amplification
Always, rTaq Plus DNA Polymerase should be added last to the mixture
High concentration of enzyme or template may lead to smearing of PCR products

2. Set PCR cycling as follows :

Initial denature at 95°C : 3 min

		1-2 kbp	3-4 kpb	5-10 kbp
Denature	95°C	10 sec	10 sec	20 sec
Anneal	Tm-4°C	10 sec	10 sec	20 sec
Extend	72°C	10 sec	20 sec	10 sec /kbp

25-35 PCR cycles

Trouble-Shooting

1. No products
   - Confirm your template is intact : Try another reaction with a result assured primer pair and templates
   - Be sure all the component are correctly added and working well : Sometimes low graded dNTP may inhibit the reaction, and degraded primers can result in low sized PCR fragments
2. Smear bands or smeared background
   - Reduce template concentration : High concentration of template can lead to smearing of PCR products. Generally, 1-10 ng of plasmid DNA and 10-100 ng of genomic DNA are working well
   - Reduce enzyme concentration in the reaction
   - Reduce elongation time
   - Increase annealing temperature
   - Set up a reaction mix on ice
3. Non-specific bands
   - Increase annealing temperature
   - Consider using PCR additives, like 1-2% DMSO and 0.5-1x Q buffer
   - Confirm specificity of your primers
4. Low yield
   - Increase enzyme concentration in the reaction
   - Increase PCR cycle number
   - Be sure appropriate concentration of your template is added