For research use only

ISO9001

Tth DNA Polymerase

Product Description	Quantity	Cat. No.	Remarks
Tth DNA Polymerase	500 unit	EBT-1016	5 unit/μl
	500 unit	EBT-1017	with 1 ml dNTP mix

Description

Tth DNA polymerase is a recombinant form of the enzyme obtained from the thermophilic eubacterium *Thermus thermophilus* expressed in *E.coli*. It is a highly processive DNA polymerase lacking 3′→5′ exonuclease activity. Tth DNA Polymerase catalyzes the polymerization of nucleotides into duplex DNA in the 5′→3′ direction in the presence of Mg^{2+}.

Tth DNA polymerase has a very efficient intrinsic reverse transcriptase (RT) activity in the presence of Mn^{2+} ion. This RT activity is not associated with RNase H activity. The ability of Tth DNA polymerase to catalyze the polymerization of DNA, using RNA templates at high temperature, minimizes the problems encountered with strong secondary structures in RNA, since they are unstable at higher reaction temperatures. Tth DNA polymerase is resistant to prolonged incubation at 95℃.

Storage Buffer

5 unit/μl in 20 mM Tris-HCl, pH8.0, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween 20, and 50% Glycerol.

Unit Definition

One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nmole of dNTP into an acid-insoluble form in 30 min at 72℃.

10x PCR Reaction Buffer w/ $MgCl_2$

500 mM Tris-HCl, pH9.0, 160 mM $(NH_4)_2SO_4$, 25 mM $MgCl_2$, 1% Triton X-100, 1 mg/ml BSA.

Optimization of [Mg^{2+}] and [Mn^{2+}]

Magnesium and manganese ion concentrations should be optimized for DNA synthesis. The optimal concentration of each metal cation is highly dependent on the dNTP concentration, templates, primers, and protocol used. For many templates, the optimal concentration of magnesium ions using Tth DNA Polymerase is approximately 1.5 mM if the concentration of each dNTP in the reaction is 0.2 mM. If used with 1.5 mM Mg^{2+}, the optimal concentration of Mn^{2+} for RNA-dependent DNA synthesis is approximately 0.5 mM for many templates. For an RT-PCR reaction, we recommend 2.5 mM Mg^{2+} and 0.5 mM Mn^{2+} for most templates.

QC tests

Activity, SDS-PAGE purity, performance tests.

Storage Condition

Store at -20℃.

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Standard Protocol

1. Prepare 50 μl PCR solution as follows:

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

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

*Note : Always, Tth DNA Polymerase should be added last to the mixture

2. Set PCR cycling as follows :

Initial denature at 95°C : 3 min

		<1 kbp	1-3 kpb
Denature	95°C	30 sec	30 sec
Anneal	Tm-4°C	30 sec	30 sec
Extend	72°C	45 sec	30-60 sec/kbp

25-40 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 is working well
   - Increase annealing temperature
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