TthPlus DNA Polymerase

CAT. NO. DP-015-0050, 500 units, 5 units/μl

APPLICATION

- Taq enzyme having both reverse transcription and DNA amplification activity, excellent for one step RT-PCR and Real-Time Quantitative PCR;
- for high temperature reverse transcription that allows highly specific priming of cDNA synthesis and overcomes problems with secondary structure.

DESCRIPTION

TthPlus DNA polymerase is isolated from the Thermus thermophilus strain. TthPlus DNA polymerase is a single 92 kDa polypeptide showing a 5'-3' exonuclease activity but lacking 3'-5' exonuclease activity. It catalyzes the polymerization of nucleotides into double-stranded DNA in the presence of $MgCl_2$. Its efficiency has been shown more particularly on large DNA fragments up to 12 kb (using lambda phage DNA as a template). TthPlus DNA polymerase is also capable of catalyzing the polymerization of DNA using an RNA template in the presence of Mn^{2+}. The ability of TthPlus DNA polymerase to reverse transcribe at elevated temperatures (70°C) minimizes the problems encountered with strong secondary structures in RNA since they are unstable at higher reaction temperatures. Higher temperatures also result in increased specificity of primer hybridization and extension. In coupled RT/PCR assays, TthPlus is about 50-100 times more efficient than regular Taq DNA polymerase.

TthPlus DNA polymerase is delivered with a one-tube 10x buffer. Both reactions (RT and PCR) are carried out in the same buffer and same tube with the same enzyme (TthPlus). The 10 x one tube buffer does not contain Mn(OAc). Mn(OAc) is provided extra and have to be added at a final concentration of 3.5 mM.

CONCENTRATION

5 units/μl

FEATURES

- thermostable
- DNA polymerase activity in the presence of $MgCl_2$
- reverse transcriptase activity in the presence of $MnCl_2$
- reverse transcription at elevated temperature minimizing secondary structure problems

UNIT DEFINITION

One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTPs into acid-insoluble form in 30 minutes at 72°C under the following reaction conditions: 25 mM TAPS buffer (Tris-(hydroxymethyl)-methyl- amino-propanesulfonic acid, sodium salt) pH 9.3 (25°C), 50 mM KCl, 2 mM $MgCl_2$, 1 mM β-mercaptoethanol, 200 μM dNTPs and 10 μg of calf thymus DNA in a final reaction volume of 50 μl.

STORAGE BUFFER

10 mM K-phosphate buffer pH 7.0 (25°C), 100 mM NaCl, 0.5 mM EDTA, 1 mM DTT, 50% glycerol (v/v), 0,1 mg/ml BSA.

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STORAGE TEMPERATURE

Store TthPlus DNA polymerase, preferably at -20°C, in a constant temperature freezer.

COMPONENT

- TthPlus DNA polymerase, 500 units
- 10 x OneTube buffer, 1 ml
- 50 mM $Mn(OAc)_2$, 0.75 ml

Note: The optimal experimental conditions depend on the system used and they should be individually determined. The Mn^{2+} concentrations and the enzyme amount are the limiting factors for an accurate result. Traditionally 5 units of enzyme and a Mn-acetate concentration of 3.5 mM are used for the reverse transcription in a final 50 µl reaction volume.

EXPERIMENTAL PROTOCOL:

Reaction Components	*Final Concentration (for 25 µl assay)*
10 x OneTube buffer	**1 x**
Mn(OAc)2	**3.5 mM (titration at 1 mM, 2.0 mM, 3.5 mM, and 4.5 mM)**
Nucleotide Mix	**0.3 mM dATP, dCTP, dGTP, 0.6 mM dUTP**
Forward and reverse primer	**7.5 pmole each**
TaqMan Probe (FAM/TAMRA)	**3.4 pmole**
TthPlus DNA polymerase	**2.5-5.0 U**

Standard Temperature cycling Program					
ABI PRISM 7000 Sequence Detection System (Applied Biosystems)					
	RT	**Hold**	**Cycle**		**Hold**
Temperature (ºC)	**59**	**95**	**95**	**59**	**25**
Time (m:s)	**60**	**10:00**	**00:30**	**01:30**	
Cycles			**40**		

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