TthPlus™ DNA Polymerase

Products	Cat #	Pack Size
TthPlus™ DNA Polymerase	GC-003-0250	250 u
TthPlus™ DNA Polymerase	GC-003-0500	500 u
TthPlus™ DNA Polymerase	GC-003-1000	1000 u
TthPlus™ DNA Polymerase	GC-003-5000	5000 u

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 efficiancy 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 a RNA template** in the presence of $MnCl_2$. 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 specificy of primer hybridization and extension. **In coupled RT/PCR assays**, TthPlus™ is about **50-100 times more efficient** than Taq DNA polymerase.

TthPlus™ DNA polymerase is delivered with 10 x RT-buffer, 5 x amplification-buffer and separate $MnCl_2$ (25 mM) and $MgCl_2$ (50 mM) solutions. The 5x amplification-buffer contains EGTA, which binds/neutalizes Mn^{2+} from the RT-reaction. Therefore after RT it is not necessary to change the buffers. For subsequent amplification we recommend to use BioTherm™ or KlenTherm™ DNA polymerase.

FEATURES

1. thermostable
2. **DNA polymerase activity** in the presence of $MgCl_2$
3. **reverse transcriptase activity** in the presence of $MnCl_2$
4. **reverse transcription at elevated temperature** minimising secondary structure problems

CONCENTRATION

5 units/µl

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 .-mer-captoethanol, 200 µM dNTPs and 10 µg of calf thymus DNA in a final reaction volume of 50 µl.

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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

STORAGE TEMPERATURE

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

10 x REACTION BUFER

670 mM Tris-HCl pH 8.8 (25°C), 166 mM $(NH_4)_2SO_4$, 0.1% Tween 20

5 x AMPLIFICATION BUFFER

335 mM Tris-HCl pH 8.8 (25°C), 83 mM $(NH_4)_2SO_4$, 3.75 mM EGTA, 25% glycerol (v/v), 0.1% Tween 20

EXTRA SOLUTIONS

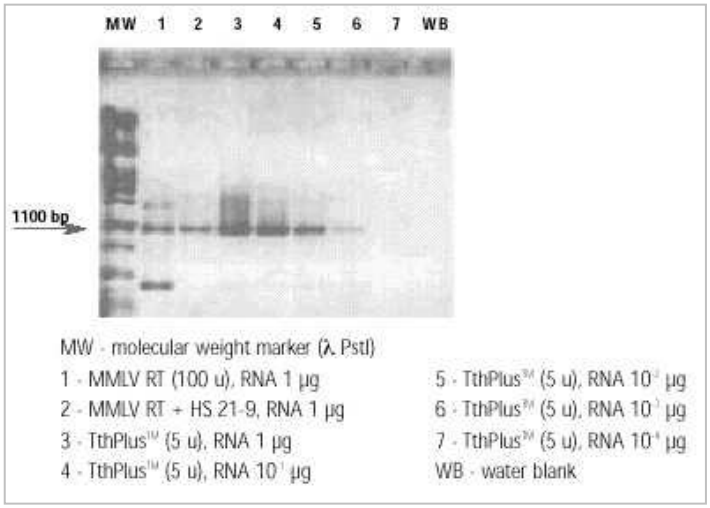
25 mM $MnCl_2$

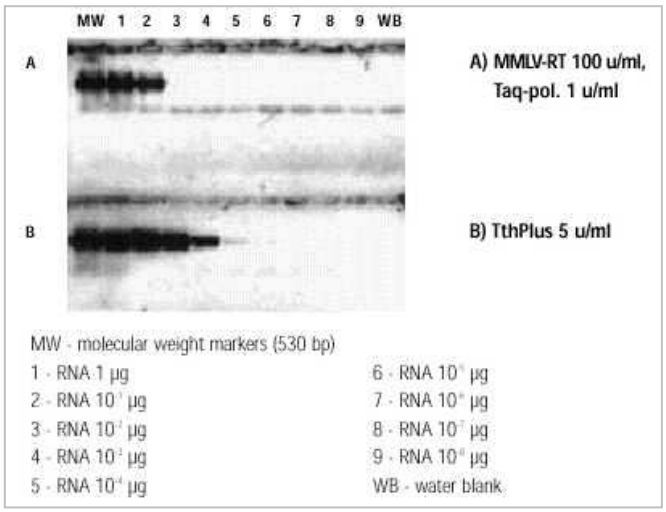
50 mM $MgCl_2$

The optimal experimental conditions depend on the system used and they should be individually determined. The Mg^{2+} or Mn^{2+} concentrations and the enzyme amount are the limiting factors for an accurate result. Tra-ditionally 5 units of enzyme and a $MnCl_2$ concentration of 1 mM are used for the reverse transcription in a final 50 µl reaction volume. For the amplification 2.5 $MgCl_2$ concentration of 1.5 mM are used for a final reaction volume of 50 µl.

Comparison of sensitivity of RT-PCR with TthPlus™ DNA polymerase and MMLV-RT

Comparison of RT-PCR with ***TthPlus™ DNA polymerase*** *and* ***MMLV-RT*** *in 16S-rRNA*





10 x ONE-TUBE BUFFER

500 Mm bicine-KOH pH 8.3, 1 M KOAc pH 7.5, 30% glycerol

EXTRA SOLUTION

50 mM $Mn/OAc)_2$

VARIOUS CONDITIONS FOR RT-PCR

Two different buffer system can be used for RT-PCR with TthPlus DNA polymerase. The first system for Tth DNA polymerase consists of 4 buffers: 1. reverse transciption (RT) buffer, 2. PCR (amplification buffer), 3. $MnCl_2$ (supplement for RT buffer), 4. $MgCl_2$ (supplement for PCR buffer). The reaction has to be carried out in two steps: RT and PCR in two different vials.

The second buffer system is so-called one-tube buffer (10x) for one-step RT-PCR. Both reactions (RT and PCR) are carried out in the same buffer and the same vial. The one-tube buffer does not contain $Mn(OAc)_2$. $Mn(OAc)_2$ is provided extra and have to be added to the one-tube buffer before the experiment. The protocol to use our TthPlus DNA polymerase is described below (buffer and polymerase conditions and cycle conditions). It was worked out for real-time RT-PCR.

PROTOCOL

EXPERIMENTAL PROTOCOL

Material

- HCV Control cRNA (10,000,000; 1,000,000; 100,000; 10,000; 1,000; 100; 50; and 10 molecules per 8-well Control strip [8 tubes/strip or 0.1 ml tubes, respectively]), Lot 008
- rTth DNA Polymerase, 2.5 U/µl (Applied Biosystems; supplier ABI)
  plus:
  5x EZ-buffer (Roboscreen)
  Mn-acetate-solution, 25 mM (Roboscreen)
- TthPlus DNA Polymerase, 5 U/µl (supplier Genecraft)
  plus:
  10x One-Tube RT-PCR-buffer (supplier Genecraft)
  Mn-acetate-solution, 50 mM (supplier Genecraft)

Instruments

- ABI PRISM 7000 Sequence Detection System (Applied Biosystems)
- Rotor-Gene 2000 (Corbett Research)

REACTION CONDITIONS (BOTH INSTRUMENTS)

Reaction component	Final concentration (25 µl-Assay)
10x/5x buffer	1x
Mn-acetate solution	3.5 mM
Nucleotide mix	0.3 mM dATP, dCTP and dGTP, 0.6 mM dUTP
Forward and reverse primer	7.5 pmol each
TaqMan® probe (FAM/TAMRA)	3.4 pmol
Tth DNA Polymerase	1.5 U

CYCLER PROGRAM

Rotor-Gene	RT	Hold	Cycle		Hold
Temperature (°C)	59	95	95	59	25
Time (min:s)					
Cycles	60	10:00	00:15	01:00	∞
			45		

7000 SDS	RT	Hold	Cycle		Hold
shut off "9600 emulation" (window "instrument")					
Temperature (°C)	59	95	95	59	25
Time (min:s)					
Cycles	60	10:00	00:30	01:30	∞
			40		