

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<sub>2</sub>. 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 a RNA template** in the presence of MnCl<sub>2</sub>. 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 Taq DNA polymerase.

TthPlus™ DNA polymerase is delivered with 10 x RT-buffer, 5 x amplification-buffer and separate MnCl<sub>2</sub> (25 mM) and MgCl<sub>2</sub> (50 mM) solutions. The 5x amplification-buffer contains EGTA, which binds/neutralizes Mn<sup>2+</sup> 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<sub>2</sub>
3. **reverse transcriptase activity** in the presence of MnCl<sub>2</sub>
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<sub>2</sub>, 1 mM β-mer-captoethanol, 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

### **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<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.1% Tween 20

### **5 x AMPLIFICATION BUFFER**

335 mM Tris-HCl pH 8.8 (25°C), 83 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 3.75 mM EGTA, 25% glycerol (v/v), 0.1% Tween 20

### **EXTRA SOLUTIONS**

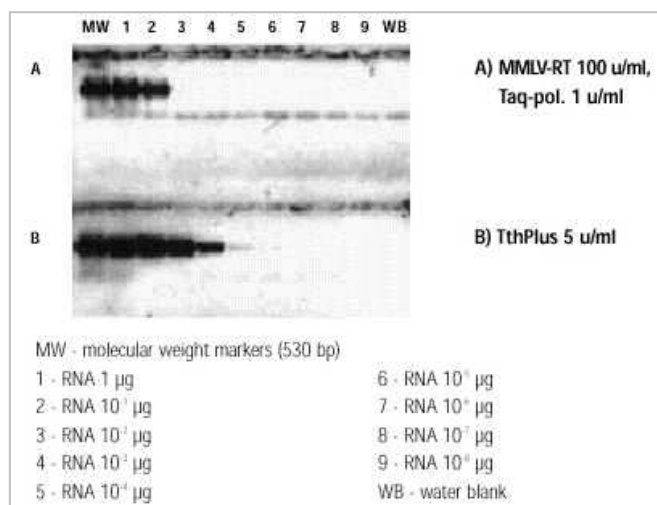
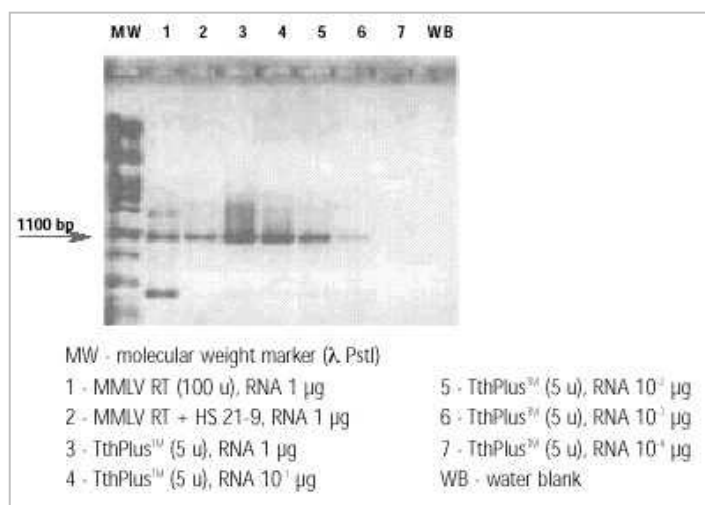
25 mM MnCl<sub>2</sub>

50 mM MgCl<sub>2</sub>

The optimal experimental conditions depend on the system used and they should be individually determined. The Mg<sup>2+</sup> or Mn<sup>2+</sup> concentrations and the enzyme amount are the limiting factors for an accurate result. Traditionally 5 units of enzyme and a MnCl<sub>2</sub> concentration of 1 mM are used for the reverse transcription in a final 50 µl reaction volume. For the amplification 2.5 MgCl<sub>2</sub> 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)<sub>2</sub>

### **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 transcription (RT) buffer, 2. PCR (amplification buffer), 3. MnCl<sub>2</sub> (supplement for RT buffer), 4. MgCl<sub>2</sub> (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)<sub>2</sub>. Mn(OAc)<sub>2</sub> 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	REACTION CONDITIONS (BOTH INSTRUMENTS)																																		
<b>Material</b>	<b>Reaction component</b> / <b>Final concentration (25 µl-Assay)</b>																																		
<ul style="list-style-type: none"> <li>■ 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</li> <li>■ rTth DNA Polymerase, 2.5 U/µl (Applied Biosystems; supplier ABI) plus: 5x EZ-buffer (Roboscreen) Mn-acetate-solution, 25 mM (Roboscreen)</li> <li>■ TthPlus DNA Polymerase, 5 U/µl (supplier Genecraft) plus: 10x One-Tube RT-PCR-buffer (supplier Genecraft) Mn-acetate-solution, 50 mM (supplier Genecraft)</li> </ul>	<table border="1"> <tr> <td>10x/5x buffer</td> <td colspan="4">1x</td> </tr> <tr> <td>Mn-acetate solution</td> <td colspan="4">3.5 mM</td> </tr> <tr> <td>Nucleotide mix</td> <td colspan="4">0.3 mM dATP, dCTP and dGTP, 0.6 mM dUTP</td> </tr> <tr> <td>Forward and reverse primer</td> <td colspan="4">7.5 pmol each</td> </tr> <tr> <td>TaqMan® probe (FAM/TAMRA)</td> <td colspan="4">3.4 pmol</td> </tr> <tr> <td>Tth DNA Polymerase</td> <td colspan="4">1.5 U</td> </tr> </table>					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			
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<ul style="list-style-type: none"> <li>■ ABI PRISM 7000 Sequence Detection System (Applied Biosystems)</li> <li>■ Rotor-Gene 2000 (Corbett Research)</li> </ul>	<table border="1"> <thead> <tr> <th>Rotor-Gene</th> <th>RT</th> <th>Hold</th> <th colspan="2">Cycle</th> <th>Hold</th> </tr> </thead> <tbody> <tr> <td>Temperature (°C)</td> <td>59</td> <td>95</td> <td>95</td> <td>59</td> <td>25</td> </tr> <tr> <td>Time (min:s)</td> <td></td> <td></td> <td>00:15</td> <td>01:00</td> <td>∞</td> </tr> <tr> <td>Cycles</td> <td>60</td> <td>10:00</td> <td colspan="2">45</td> <td></td> </tr> </tbody> </table>					Rotor-Gene	RT	Hold	Cycle		Hold	Temperature (°C)	59	95	95	59	25	Time (min:s)			00:15	01:00	∞	Cycles	60	10:00	45								
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