

## DATA QUALITY SHEET



### **TthPlus DNA Polymerase**

FOR RESEARCH USE ONLY

*Cat. GC-002-0100, GC-002-0250, GC-002-0500, GC-002-1000, GC-002-5000*

#### **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.

#### **CONCENTRATION**

5 units/μl.

#### **UNIT DEFINITION**

One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTP's into acid-insoluble form in 30 minutes at 72°C under the assay conditions (25 mM TAPS (tris-(hydroxymethyl)-methyl-amino-propanesulfonic acid, sodium salt) pH 9.3 (at 25°C); 50 mM KCl; 2 mM MgCl<sub>2</sub>; 1 mM β-mercaptoethanol) and activated calf thymus DNA as substrate.

#### **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 BioTherm Polymerase below 0°C preferably at -20°C, in a constant temperature freezer.

#### **ASSOCIATED ACTIVITIES**

Endonuclease and exonuclease activities were not detectable after 2 and 1 hours incubation, respectively, of 1 μg lambda DNA and 0.22 μg of EcoRI digested lambda DNA, respectively, at 72°C in the presence of 15-20 units of BioTherm Polymerase

#### **SHELF LIFE**

2 years from date of receipt under proper storage conditions

## 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.  $MnCl_2$  (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 by our customer Roboscreen GmbH.

### buffers for two-step-PCR:

<b>10X REACTION BUFER</b>	670 mM Tris-HCl pH 8.8 (25°C), 166 mM $(NH_4)_2SO_4$ , 0.1% Tween 20
<b>5x AMPLIFICATION BUFFER</b>	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
<b>EXRTA SOLUTIONS</b>	25mMMnCl <sub>2</sub> 50 mM MgCl <sub>2</sub>

### buffers for two-step-PCR :

<b>10X ONE-TUBE BUFFER</b>	500 Mm bicine-KOH pH 8.3, 1 M KOAc pH 7.5, 30% glycerol
<b>EXTRA SOLUTION</b>	50 mM Mn/OAc <sub>2</sub>
<b>FUNCTIONAL ANALYSIS</b>	Tested functionally in a unit activity test