Triggering Reverse Transcriptase Activity of

Taq DNA Polymerase

Contributed Protocol
Contributed by Dr. Jörn Glökler, Max-Planck-Institute for Molecular Genetics Berlin, 22.06.2009

Taq DNA Polymerase Recombinant (Thermus aquaticus)

Cat. No.	Size
E2500-01	200 units
E2500-02	1000 units
E2500-03	5000 units
E2500-04	500 units

Taq DNA Polymerase Native (Thermus aquaticus)

Cat. No.	Size
E2504-01	200 units
E2504-02	1000 units
E2504-03	5000 units
E2504-04	500 units

Unit Definition: One unit is the amount of enzyme required to incorporate 10 nmoles of total deoxyribonucleotide into acid-insoluble form in 30 min at 60°C.

Storage Conditions:
Store at -20°C

This protocol describes triggering Reverse Transcriptase activity of Taq DNA polymerase for conducting one step RT/ PCR reactions.

Similar to Tth DNA Polymerase (2), Reverse Transcriptase activity of Taq DNA Polymerase can be invoked by using Mn^{2+}- instead of Mg^{2+}-ions in the reaction buffer. Grabko *et al.* (1) suggested an optimized protocol for performing RT/PCR reactions using Taq DNA Polymerase as the sole enzyme for conducting both the RT and PCR reaction steps. The authors demonstrated successful amplification of a 960 bp fragment starting from a completely DNA-free poliovirus RNA sample. Starting from 5 ng RNA template, 20 µg DNA were obtained (1). Their protocol can be adopted for this enzyme preparation as follows:

RT-PCR PROTOCOL

Reverse Transcription using Taq DNA Polymerase
Concentrations are given as final concentrations.

Template RNA	x µl (5 – 200 ng RNA)
Reverse Primer	20 pmol
Tris-HCl, pH 8.8	67 mM
$MnCl_2$	2 mM
dNTPs	250 µM
$(NH_4)_2SO_4$	16.6 mM
Tween-20	0.01 % (v/v)
Taq DNA Polymerase	5 U
H_2O sterile	ad 20 µl

RT Reaction Conditions:
3 min at 56°C for primer annealing (primer dependent temperature)
10 min at 70°C reverse transcription (depends on template length)

Follow-up PCR Reaction

Add 80 µl buffer containing

EGTA	0.75 mM
PCR Buffer A (without $MgCl_2$)	8 µl
Reverse Primer	80 pmol
Forward Primer	100 pmol
dNTPs	200 µM
$MgCl_2$	2 mM

Perform PCR program of choice.
Sensitivity: ~ 10^{-4} RNAs

References:

1. *Grabko, V. et al. (1996) FEBS Letters 387, 189-92.*
2. *Myers, T. W., Gelfand, D. H. (1991) Biochemistry 30, 7661-6.*