For life science research only.
Not for use in diagnostic procedures.

Tth DNA Polymerase

From *Thermus thermophilus*, recombinant (*E. coli*)
Deoxynucleoside-triphosphate: DNA deoxynucleotidyltransferase, *EC 2.7.7.7* (DNA-directed)

Cat. No. 11 480 022 001 500 U (2× 250 U)

Version 20
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Store at −15 to −25°C

1. What this Product Does

Product Overview

Vial	Content
Tth DNA Polymerase	500 U Enzyme storage buffer: 10 mM Tris-HCl, 1 mM dithiothreitol, 0.1 mM EDTA, 300 mM KCl, 0.1% Triton X-100 (v/v), 50% glycerol (v/v), pH 7.5 (+25°C)
PCR buffer, 10× conc.	100 mM Tris-HCl, 15 mM $MgCl_2$, 1 M KCl, 500 µg/ml bovine serum albumin (BSA), 0.5% Tween 20 (v/v), pH 8.9 (+25°C)
RT-PCR buffer, One-Step reaction, 5× conc.	250 mM bicine/KOH, pH 8.2 (+25°C), 575 mM K-acetate, 40% glycerol (v/v)
Mn $(OAc)_2$ solution	25 mM

Product Description

Tth DNA Polymerase (2) is isolated from the thermophilic eubacterium *Thermus thermophilus*. The enzyme preparation is free of nonspecific DNases and RNases according to current Quality Control procedures. It is a highly processive 5'-3' DNA polymerase which lacks 3'-5' exonuclease activity.

Enzyme Properties

Tth DNA Polymerase activity is resistant to prolonged incubations at high temperatures (+95°C), and can therefore be used for PCR (polymerase chain reaction) amplification (3,4).
In the presence of manganese ions Tth DNA Polymerase has a very efficient intrinsic reverse transcriptase (RT) activity (5), which is much higher than the activity reported for *E. coli* DNA polymerase (6) and Taq DNA polymerase (7).

Volume activity	5 U/µl, determined in the assay on activated DNA described under *Unit Assay.*
Optimal enzyme concentration	0.5 to 5 U per reaction for PCR
Standard enzyme concentration	2.5 U per reaction for PCR
Optimal pH	around 9 (adjusted at +25°C)
Optimal temperatures	around +75°C
Optimal Mg^{2+} concentrations	1 to 10 mM
Standard Mg^{2+} concentration	1.5 mM
PCR product size	Optimized for at least up to 1,000 bp in a RT-PCR reaction.
Incorporation of modified nucleotides	Accepts modified desoxyribonucleoside triphosphates as substrates, and can be used to label DNA fragments with modified deoxynucleotides labeled with digoxigenin, fluorescein or biotin during PCR.
RNase H activity	No association with RNase H activity.
Thermostability	The high thermostability of Tth DNA Polymerase overcomes the problem typically associated with the high degree of secondary structure present in RNA (8).

RT-PCR

RT-PCR is an important technique for detection, quantification and cloning of mRNA as well as for gene expression studies. The ability to perform a coupled reverse transcription and amplification reaction using identical buffer conditions in one tube means that Tth DNA Polymerase reduces the handling steps and thus the potential risks associated with contamination of a RT-PCR reaction.

Storage and Stability

Stable at −15 to −25°C until the expiration date printed on the label.

Applications

- Polymerase Chain Reaction (PCR)
- RT-PCR
- DNA Labeling reactions

2. How to Use this Product

2.1 Before You Begin

General Considerations

Optimal reaction conditions (incubation temperatures, concentration of Tth DNA Polymerase, template DNA or RNA concentration, primers, Mg^{2+}) depend on the respective target sequence, and have to be determined individually.

RNA Preparations

For high quality eukaryotic mRNA preparations it is necessary to minimize the activity of RNases released during cell lysis by using inhibitors of RNases or methods that disrupt cells and simultaneously inactivate RNases. A good overview of appropriate methods is given in references 11 and 12.

For the isolation of mRNA or total RNA it is recommended to use the mRNA Isolation Kit* and the TriPure Isolation Reagent*. Furthermore any contamination with RNases from other potential sources like glassware, plastic ware, reagent solutions and the hands of the investigator have to be avoided.

Reference 11 list several measures to avoid problems with RNase contamination.

In a typical mammalian cell only 1 to 5% of the total cellular RNA will be mRNA. However, most eucaryotic mRNAs possess a tail of polyadenylic acid residues at their 3'-end that is generally long enough to allow mRNA purification by affinity chromatography on oligo(dT) cellulose or by use of oligo$(dT)_{20}$, biotin-labeled, and Streptavidin Magnetic Particles*.

Amount of RNA

Do not add more than 1 µg of total RNA.

Length of the RT-PCR Products

Under optimal conditions, Tth DNA Polymerase can reverse transcribe and amplify fragments up to 2 to 3 kb. To guarantee efficient amplification without optimization, the fragment length should be smaller than 1 kb.

Manganese Concentration

The optimal Mn^{2+} concentration for RT-PCR ranges from 1 to 4 mM and depends on the primer pair used.

Carryover Contamination

For the incorporation of dUTP instead of dTTP, Roche recommends increasing the concentration of dUTP to 500 µM to obtain higher yield. Higher concentrations of Mn^{2+} and more units of Tth DNA Polymerase will also improve the RT-PCR performance.

2.2 One-Step RT-PCR Protocol

General Consideration for One-Step RT-PCR

For coupled RT-PCR amplification, meaning one-step reverse transcription and amplification, the concentration of Mn^{2+} needs to be determined by testing Mn^{2+} concentrations from 1 to 4 mM for each primer set used. Reagents, such as dNTPs, template DNA and primer can chelate manganese ions. For cDNA synthesis the use of a specific primer is recommended; hexamer primers or oligo (dT) primers are not suitable. dUTP can be used instead of dTTP to perform carryover prevention. The efficiency of the reaction can be improved by using 500 µM dUTP and 200 µM (each) of the other dNTP

Preparation of Reaction Mixes

It is recommended to prepare two master mixes:

Step	Action
❶	Briefly centrifuge all reagents before starting.
❷	• Prepare two mixes of reagents in sterile microcentrifuge tubes (on ice): • **Mix 1 (for one reaction**)

Reagents	Volume	Final conc.
Water, PCR Grade	add up to 25 µl	
dATP (10 mM)	1.5 µl	300 µM
dCTP (10 mM)	1.5 µl	300 µM
dGTP (10 mM)	1.5 µl	300 µM
dTTP (10 mM)	1.5 µl	300 µM
Forward primer 1	variable	450 nM
Reverse primer 2	variable	450 nM
Template RNA	variable	up to 1 µg
Final volume	**25 µl**	

• Mix 2 (for one reaction)

Reagents	Volume	Final conc.
Water, PCR Grade	add up to 25 µl	
5× RT-PCR buffer	10 µl	1×
Mn $(OAc)_2$, 25 mM	5 µl	2.5 mM
Tth DNA Polymerase	1 µl	5 U/reaction*
Final volume	**25 µl**	

*It is recommended to use 5 U per reaction. Depending on primers and target, the use of 2.5 U per reaction might give better results.

Step	Action
❸	• Combine Mix 1 and Mix 2 in a thin-walled PCR tube (on ice). • Gently vortex the mixture to produce a homogeneous reaction, then centrifuge briefly to collect the sample at the bottom of the tube. • Continue to thermal cycling immediately. Carefully overlay the reaction with mineral oil if required by your type of thermal cycler.

Thermal Cycling

Place samples in the thermal cycler, and start cycling using the thermal profiles below.

The thermal profiles were developed for the Perkin Elmer GeneAmp PCR System 9600. Other thermal cyclers may require different profiles.

Step	Temperature	Time	Cycle No.
RT Reaction	60 to 70°C	30 min	1 ×
Denaturation	94°C	1 min	1 ×
Denaturation Annealing Elongation	94°C 50 to 70°C* 72°C	30 sec 30 sec 45 sec	10 ×
Denaturation Annealing Elongation	94°C 50 to 70°C* 72°C	30 sec 30 sec 45 sec, add to each cycle 5 sec (cycle elongation)	20 to 30 ×**
Final Elongation	72°C	7 min	1 ×

Analyze samples on a 1 to 2% agarose gel.

*The annealing temperature depends on the melting temperature of the primer used.

**The cycle number depends on the abundance (copy number) of the respective RNA.

2.3 Two-Step RT-PCR Protocol

Application for Two-Step RT-PCR

Tth DNA Polymerase also can be used in a Two-Step RT-PCR reaction setup. A protocol and thermal cycling profile is outlined below. Please note that all relevant buffers for this application are not provided with this product.

Preparation of Master Mixes

It is recommended to prepare a Master Mix for setting up multiple reactions. The master mix typically contains all of the components needed for the respective number of reactions.

Step	Action
❶	Briefly centrifuge all reagents before starting.
❷	Add to a sterile RNAse- and DNase-free microcentrifuge tubes (on ice):

Reagents	Volume	Final conc.
Water, PCR Grade	add up to 20 µl	
10× Rev. Transcription Buffer (100 mM Tris-HCl pH 8.9, 900 mM KCl)	2 µl	1 ×
$MnCl_2$ (9 mM)	2 µl	0.9 mM
dATP (10 mM)	0.4 µl	200 µM
dCTP (10 mM)	0.4 µl	200 µM
dGTP (10 mM)	0.4 µl	200 µM
dTTP (10 mM)	0.4 µl	200 µM
Reverse primer	variable	750 nM
Template RNA	variable	up to 200 ng
Tth DNA Polymerase	0.8 µl	4 U
Final volume	**20 µl**	

Step	Action
❸	Incubate for 10 to 30 min at +60 to +70°C. Ⓢ The temperature depends on the 1st strand primer used; +70°C is the optimal reaction temperature for Tth DNA Polymerase however RNA degradation increases at higher temperatures.
❹	Add 80 µl of freshly preparaed PCR mix (see below) to the reverse transcription reaction (total volume 100 µl) at +15 to +25°C.

Reagents	Volume	Final conc.
Water, PCR Grade	add up to 80 µl	
10× PCR buffer	8 µl	0.8×
EGTA, 7.5 mM	10 µl	0.75 nM
Forward primer	variable	150 nM

Step	Action
❺	• Mix and centrifuge briefly to collect the sample at the bottom of the tube. • Continue to thermal cycling immediately. Ⓢ Carefully overlay the reaction with mineral oil if required by your type of thermal cycler.

Thermal Cycling

Place samples in the thermal cycler, and start cycling using the thermal profiles below.

Ⓢ The thermal profiles were developed for the Perkin Elmer GeneAmp PCR System 9600. Other thermal cyclers may require different profiles.

Step	Temperature	Time	Cycle No.
Initial Denaturation	94°C	1 min	1 ×
Denaturation Annealing Elongation	94°C 50 to 70°C* 72°C	30 sec 30 sec 45 sec	10 ×
Denaturation Annealing Elongation	94°C 50 to 70°C* 72°C	30 sec 30 sec 45 sec, add to each cycle 5 sec (cycle elongation)	20 to 30 ×**
Final Elongation	72°C	7 min	1 ×

Analyze samples on a 1 to 2% agarose gel.

*The annealing temperature depends on the melting temperature of the primer used.

**The cycle number depends on the abundance (copy number) of the respective RNA.

2.4 PCR Protocol

Preparation of Master Mixes

It is recommended to prepare two master mixes:

Step	Action
❶	Briefly centrifuge all reagents before starting.
❷	• Prepare two mixes of reagents in sterile microcentrifuge tubes (on ice): • **Mix 1 (for one reaction**)

Reagents	Volume	Final conc.
Water, PCR Grade	add up to 50 µl	
dATP (10 mM)	2 µl	200 µM
dCTP (10 mM)	2 µl	200 µM
dGTP (10 mM)	2 µl	200 µM
dTTP (10 mM)	2 µl	200 µM
Forward primer 1	variable	400 nM
Reverse primer 2	variable	400 nM
Template DNA	variable	up to 0.5 µg
Final volume	**50 µl**	

Step	Action
	• **Mix 2 (for one reaction)**

Reagents	Volume	Final conc.
Water, PCR Grade	add up to 50 µl	
10× PCR buffer with Mg	10 µl	1×
Tth DNA Polymerase	0.5 µl	2.5 U/ reaction*
Final volume	**50 µl**	

*It is recommended to use 5 U per reaction. Depending on primers and target, the use of 2.5 U per reaction might give better results.

Step	Action
❸	• Combine Mix 1 and Mix 2 in a thin-walled PCR tube (on ice). • Gently vortex the mixture to produce a homogeneous reaction, then centrifuge briefly to collect the sample at the bottom of the tube. • Continue to thermal cycling immediately. Ⓢ Carefully overlay the reaction with mineral oil if required by your type of thermal cycler.

Thermal Cycling

Place samples in the thermal cycler, and start cycling using the thermal profiles below.

Ⓢ The thermal profiles were developed for the Perkin Elmer GeneAmp PCR System 9600. Other thermal cyclers may require different profiles.

Step	Temperature	Time	Cycle No.
Initial Denaturation	94°C	1 min	1 ×
Denaturation Annealing Elongation	94°C 50 to 70°C* 72°C	30 sec 30 sec 45 sec**	10 ×
Denaturation Annealing Elongation	94°C 50 to 70°C* 72°C	30 sec 30 sec 45 sec**, add to each cycle 5 sec (cycle elongation)	20 ×
Final Elongation	72°C	7 min	1 ×

Analyze samples on a 1 to 2% agarose gel.

*The annealing temperature depends on the melting temperature of the primer used.

**The cycle number depends on the abundance (copy number) of the respective RNA.

2.5 Unit Assay

Unit Definition

One unit of Tth DNA Polymerase is defined as the amount of enzyme which catalyses the incorporation of 10 nmol total dNTPs into acid precipitable DNA within 30 min at +70°C under the assay conditions stated above.

Assay on Activated DNA

Incubation buffer for assay on activated DNA:

67 mM Tris-HCl, pH 8.8 (+25°C), 16.6 mM $(NH_4)_2SO_4$, 6.7 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 0.2 mM dATP, dCTP, dGTP, dTTP each.

Incubation procedure: 12.5 µg activated (1) herring sperm DNA and 0.1 µCi [α^{32}P] dCTP are incubated with 0.01 to 0.1 units Tth DNA Polymerase in 50 µl incubation buffer with a paraffin oil overlay at +70°C for 30 min.

The amount of incorporated dNTPs is determined by trichloroacetic acid precipitation followed by scintillation counting.

2.6 Quality Control

For lot-specific certificates of analysis, see section, **Contact and Support**.

References

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6 Jones, M.D. & Foulkes, N.S. (1989) *Nucleic Acids, Res.* **17**, 8387 – 8388.
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11 Sambrook, J. *et al.* (1989) Molecular Cloning, A Laboratory Manual, Second Edition, (Cold Harbor Laboratory) Part 7 und 8.
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Changes to Previous Versions

- Update of the chapter Quality Control.

3. Supplementary Information

3.1 Conventions

Text Conventions

To make information consistent and understandable, the following text conventions are used in this document:

Text Conventions	Use
Numbered Instructions labeled ❶, ❷, *etc.*	Steps in a procedure that must be performed in the order listed.
Asterisk *	Denotes a product available from Roche Diagnostics.

Symbols

In this document, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.

Ordering Information

Product	Pack Size	Cat. No.
AptaTaq Fast DNA Polymerase	100 U 1,000 U	06 879 110 001 06 879 128 001
Taq DNA Polymerase, 5 U/µl	100 U 500 U 4× 250 U 10× 250 U 20× 250 U	11 146 165 001 11 146 173 001 11 418 432 001 11 596 594 001 11 435 094 001
PCR Core Kit^{PLUS}	1 kit	11 585 541 001
PCR Core Kit	1 kit	11 578 553 001
PCR Master	1 kit	11 636 103 001
Expand High Fidelity PCR System	100 U 2× 250 U 10× 250 U	11 732 641 001 11 732 650 001 11 759 078 001
Expand High Fidelity^{PLUS} PCR System	2× 250 U 10× 250 U	03 300 226 001 03 300 234 001
Expand 20 kb^{PLUS} PCR System	200 U	11 811 002 001
Expand Long Range, dNTPack	175 U 700 U 3,500 U	04 829 034 001 04 829 042 001 04 829 069 001
High Fidelity PCR Master	1 kit	12 140 314 001
FastStart Taq DNA Polymerase, 5 U/µl	100 U 500 U 4× 250 U 10× 250 U 20× 250 U	12 032 902 001 12 032 929 001 12 032 937 001 12 032 945 001 12 032 953 001
FastStart High Fidelity PCR System	2× 250 U 10× 250 U	03 553 400 001 03 553 361 001
Pwo SuperYield DNA Polymerase	100 U 2× 250 U	04 340 868 001 04 340 850 001
Pwo Master	1 kit	03 789 403 001
Pwo DNA Polymerase	100 U 2× 250 U	11 644 947 001 11 644 955 001
GC-RICH PCR System	100 U	12 140 306 001
Special Nucleotides Mixes		
Deoxynucleoside Triphosphate Set, PCR Grade, sodium salt	4× 25 µmol 4× 125 µmol	11 969 064 001 03 622 614 001
Deoxynucleoside Triphosphate Set, lithium salt	4× 10 µmol 40 × 10 µmol	11 277 049 001 11 922 505 001
PCR Nucleotide Mix	200 µl 2,000 µl	11 581 295 001 11 814 362 001
PCR Nucleotide Mix^{PLUS}	2× 100 µl	11 888 412 001
Additional Reagents		
DIG-11-dUTP, alkali-stable	25 nmol (25 µl) 125 nmol (125 µl) 5 × 125 nmol (5 × 125 µl)	11 093 088 910 11 558 706 910 11 570 013 910
DIG-11-dUTP, alkali-labile	25 nmol (25 µl) 125 nmol (125 µl)	11 573 152 910 11 573 179 910
Water, PCR Grade	25 ml (25 vials of 1 ml) 25 ml (1 vial of 25 ml) 100 ml (4 vials of 25 ml)	03 315 932 001 03 315 959 001 03 315 843 001
Uracil-DNA Glycosylase, heat-labile	100 units 500 units	11 775 367 001 11 775 375 001
High Pure PCR Product Purification Kit	50 spin filter 250 spin filter	11 732 668 001 11 732 676 001
mRNA Isolation Kit	Isolation of at least 70 µg of poly(A$^+$) RNA	11 741 985 001
TriPure Isolation Reagent	50 ml 200 ml	11 667 157 001 11 667 165 001
Streptavidin Magnetic Particles	2 ml 10 ml	11 641 778 001 11 641 786 001

Trademarks

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Disclaimer of License

For patent license limitations for individual products please refer to:
List of biochemical reagent products

Contact and Support

To ask questions, solve problems, suggest enhancements and report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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