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

Tth DNA Polymerase
from *Thermus thermophilus*, recombinant (*E. coli*)

Version: 22
Content Version: January 2025

Deoxynucleoside-triphosphate: DNA deoxynucleotidyltransferase, (DNA-directed)

Cat. No. 11 480 022 001	500 U 2 x 250 U, 5 U/µl

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	Tth DNA Polymerase	Enzyme storage buffer: 10 mM Tris-HCl, 1 mM dithiothreitol, 0.1 mM EDTA, 300 mM KCl, 0.2 % Tween 20 (v/v), 50% glycerol (v/v), pH 7.5 (+25°C).	2 vials, 250 U
2	Tth DNA Polymerase, PCR Buffer, 10x conc.	Buffer composition: 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).	1 vial, 1 ml
3	Tth DNA Polymerase, RT-PCR-Buffer, 5x conc.	▪ Buffer composition: 250 mM bicine/KOH, pH 8.2 (+25°C), 575 mM potassium acetate, 40% glycerol (v/v). ▪ For one-step reaction.	1 vial, 1 ml
4	Tth DNA Polymerase, Mn-Acetate Solution	25 mM	1 vial, 1 ml

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the product is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	Tth DNA Polymerase	Store at −15 to −25°C.
2	PCR Buffer, 10x conc.	
3	RT-PCR-Buffer, 5x conc.	
4	Mn-Acetate Solution	

1.3. Additional Equipment and Reagent required

Standard laboratory equipment and reagents

- Thermal block cycler
- Microcentrifuge
- Sterile RNase- and DNase-free microcentrifuge tubes
- Thin-walled PCR tubes
- Mineral oil (optional)
- 1 to 2% agarose gel

For one-step RT-PCR

- Water, PCR Grade*
- Forward primer 1
- Reverse primer 2
- PCR nucleotide mix(1)

For two-step RT-PCR

- Water, PCR Grade*
- 10x Reverse transcription buffer: 100 mM Tris-HCl, pH 8.9, 900 mM KCl
- 9 mM $MnCl_2$
- Forward primer 1
- Reverse primer 2
- PCR nucleotide mix(1)
- 7.5 mM EGTA

For PCR

- Water, PCR Grade*
- Forward primer 1
- Reverse primer 2
- PCR nucleotide mix(1)
- Optional: other polymerases(2)

(1) Such as the Deoxynucleoside Triphophate Set, sodium salt*, Deoxynucleoside Triphosphate Set, lithium salt*, PCR Nucleotide Mix*, or the PCR Nucleotide Mix^{PLUS}*.

(2) Such as the Expand High Fidelity^{PLUS} PCR System*, FastStart High Fidelity PCR System*, Pwo SuperYield DNA Polymerase*, or the GC-RICH PCR System*.

1.4. Application

Tth DNA Polymerase is used in a variety of techniques:

- PCR
- RT-PCR
- DNA labeling reactions

2. How to Use this Product

2.1. Before you Begin

Sample Materials

For template RNA, use up to 1 µg total RNA.

- 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.
- For the isolation of mRNA or total RNA, use the mRNA Isolation Kit* and the TriPure Isolation Reagent*.
- Avoid any contamination with RNases from other potential sources such as glassware, plasticware, reagent solutions, and hands.
- In a typical mammalian cell, only 1 to 5% of the total cellular RNA will be mRNA. However, most eukaryotic 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 using oligo(dT)$_{20}$, biotin-labeled, and Streptavidin Magnetic Particles*.

Mg^{2+} Concentration

1 to 10 mM (optimal)
1.5 mM (standard)

General Considerations

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

Manganese concentration

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

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, use a fragment length <1 kb.

Enzyme properties

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

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.

Prevention of Carryover Contamination

The use of Uracil-DNA Glycosylase* and dUTP allows the application of the carryover prevention technique to reduce the number of false-positive results in PCR and RT-PCR.
For the incorporation of dUTP instead of dTTP, increase 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. Protocols

One-step RT-PCR

For coupled RT-PCR amplification, also called one-step reverse transcription and amplification, determine the concentration of Mn^{2+} 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, use a specific primer; 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

Prepare two master mixes.

1. Briefly centrifuge all reagents before starting.

2. Prepare two mixes of reagents in sterile microcentrifuge tubes on ice.

   – Mix 1 (for one reaction) is shown in the table:

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

   – Mix 2 (for one reaction) is shown in the table:

Reagent	Volume [µl]	Final conc.
Water, PCR Grade*	add up to 25	–
RT-PCR-Buffer 5x conc.	10	1x
Mn-Acetate Solution, 25 mM	5.0	2.5 mM
Tth DNA Polymerase	1.0	5 U/reaction[1]
Final volume	**25**	

3. Combine Mix 1 and Mix 2 in a thin-walled PCR tube on ice.
   – Gently vortex the mixture to produce a homogeneous reaction; centrifuge briefly to collect the sample at the bottom of the tube.
   – Immediately proceed to thermal cycling.

   Carefully overlay the reaction with mineral oil if required by the type of thermal cycler.

(1) 5 U/reaction is a good starting point. Depending on the primers and target, 2.5 U/reaction may give better results.

Thermal cycling

1. Place samples in the thermal cycler, and start cycling using the thermal profiles shown in the table.

i *The following thermal profiles are an example. Different thermal cyclers may require different profiles.*

Step	Temperature [°C]	Time	Number of Cycles
RT Reaction	60 – 70	30 min	1
Pre-Incubation	94	1 min	1
Denaturation Annealing Elongation	94 50 – 70[(1)] 72	30 sec 30 sec 45 sec	10
Denaturation Annealing Elongation	94 50 – 70[(1)] 72	30 sec 30 sec 45 sec, add to each cycle 5 sec (cycle elongation)	20 – 30[(2)]
Final Elongation	72	7 min	1

2. Analyze samples on a 1 to 2% agarose gel.

(1) Annealing temperature depends on the melting temperature of the primer used.
(2) Cycle number depends on the abundance (copy number) of the respective RNA.

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 in the following sections.

i *All relevant buffers for this application are not provided with this product.*

Preparation of master mixes

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.

1. Briefly centrifuge all reagents before starting.

2. Add to sterile RNase- and DNase-free microcentrifuge tubes on ice:

Reagent	Volume [µl]	Final conc.
Water, PCR Grade*	add up to 20	–
10x Reverse transcription buffer	2	1x
$MnCl_2$ (9 mM)	2	0.9 mM
dATP (10 mM)	0.4	200 µM
dCTP (10 mM)	0.4	200 µM
dGTP (10 mM)	0.4	200 µM
dTTP (10 mM)	0.4	200 µM
Reverse primer 2	variable	750 nM
Template RNA	variable	up to 200 ng
Tth DNA Polymerase	0.8	4 U
Final volume	**20**	

3. Incubate for 10 to 30 minutes at +60 to +70°C.

i *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.*

4. Add 80 µl of freshly prepared PCR mix as shown in the table to the reverse transcription reaction (total volume 100 µl) at +15 to +25°C.

Reagent	Volume [µl]	Final conc.
Water, PCR Grade*	add up to 80	–
PCR Buffer, 10x conc.	8	0.8x
EGTA (7.5 mM)	10	0.75 nM
Forward primer 1	variable	150 nM

5. Mix and centrifuge briefly to collect the sample at the bottom of the tube.
– Immediately proceed to thermal cycling.

Carefully overlay the reaction with mineral oil if required by the type of thermal cycler.

Thermal cycling

1. Place samples in the thermal cycler, and start cycling using the thermal profiles shown in the table.

The following thermal profiles are an example. Different thermal cyclers may require different profiles.

Step	Temperature [°C]	Time	Number of Cycles
Pre-Incubation	94	1 min	1
Denaturation Annealing Elongation	94 50 – 70[1] 72	30 sec 30 sec 45 sec	10
Denaturation Annealing Elongation	94 50 – 70[1] 72	30 sec 30 sec 45 sec, add to each cycle 5 sec (cycle elongation)	20 – 30[2]
Final Elongation	72	7 min	1

2. Analyze samples on a 1 to 2% agarose gel.

(1) Annealing temperature depends on the melting temperature of the primer used.
(2) Cycle number depends on the abundance (copy number) of the respective RNA.

PCR protocol

Preparation of reaction mixes

Prepare two master mixes.

1. Briefly centrifuge all reagents before starting.

2. Prepare two mixes of reagents in sterile microcentrifuge tubes on ice.

   – Mix 1 (for one reaction) is shown in the table:

Reagent	Volume [µl]	Final conc.
Water, PCR Grade*	add up to 50	–
dATP (10 mM)	2	200 µM
dCTP (10 mM)	2	200 µM
dGTP (10 mM)	2	200 µM
dTTP (10 mM)	2	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**	

   – Mix 2 (for one reaction) is shown in the table:

Reagent	Volume [µl]	Final conc.
Water, PCR Grade*	add up to 50	–
10x PCR Buffer with Mg	10	1x
Tth DNA Polymerase	0.5	2.5 U/reaction[1]
Final volume	**50**	

3. Combine Mix 1 and Mix 2 in a thin-walled PCR tube on ice.
   – Gently vortex the mixture to produce a homogeneous reaction; centrifuge briefly to collect the sample at the bottom of the tube.
   – Immediately proceed to thermal cycling.

   Carefully overlay the reaction with mineral oil if required by the type of thermal cycler.

[1] 5 U/reaction is a good starting point. Depending on the primers and target, 2.5 U/reaction may give better results.

Thermal cycling

1. Place samples in the thermal cycler, and start cycling using the thermal profiles shown in the table.

i *The following thermal profiles are an example. Different thermal cyclers may require different profiles.*

Step	Temperature [°C]	Time	Number of Cycles
Pre-Incubation	94	1 min	1
Denaturation Annealing Elongation	94 50 – 70[1] 72	30 sec 30 sec 45 sec	10
Denaturation Annealing Elongation	94 50 – 70[1] 72	30 sec 30 sec 45 sec, add to each cycle 5 sec (cycle elongation)	20[2]
Final Elongation	72	7 min	1

2. Analyze samples on a 1 to 2% agarose gel.

(1) Annealing temperature depends on the melting temperature of the primer used.
(2) Cycle number depends on the abundance (copy number) of the respective RNA.

2.3. Parameters

EC-Number

EC 2.7.7.7

Incorporation of Modified Nucleotides

Accepts modified deoxyribonucleoside triphosphates as substrates and can be used to label DNA fragments with modified deoxynucleotides labeled with digoxigenin, fluorescein, or biotin during PCR.

Maximum Fragment Size

Optimized for ≤1,000 bp in an RT-PCR reaction.

pH Optimum

Approximately 9 (+25°C).

RNase H Activity

None

Temperature Optimum

Approximately +75°C.

Temperature Stability

The high thermostability of Tth DNA Polymerase overcomes the problem typically associated with the high degree of secondary structure present in RNA.

Unit Assay

Assay on activated DNA

Incubation buffer

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

❶ 12.5 µg activated herring sperm DNA and 0.1 µCi [α^{32}P] dCTP are incubated with 0.01 to 0.1 U Tth DNA Polymerase in 50 µl Incubation buffer with a paraffin oil overlay at +70°C for 30 minutes.

---

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

Unit Definition

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

Volume Activity

5 U/µl, determined in the assay on activated DNA described in section, **Unit Assay**.

Working Concentration

0.5 to 5 U per reaction for PCR (optimal).
2.5 U per reaction for PCR (standard).

3. Additional Information on this Product

3.1. Quality Control

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

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
ℹ *Information Note: Additional information about the current topic or procedure.*	
⚠ ***Important Note: Information critical to the success of the current procedure or use of the product.***	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.
Exchange of Triton X-100 with Tween 20 according to REACH regulatory.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
PCR Nucleotide Mix	200 µl, 500 reactions of 20 µl final reaction volume	11 581 295 001
	5 x 200 µl, 2,500 reactions of 20 µl final reaction volume.	04 638 956 001
	10 x 200 µl, 5,000 reactions of 20 µl final reaction volume.	11 814 362 001
PCR Nucleotide Mix^{PLUS}	2 x 100 µl, 200 PCR reactions in 50 µl	11 888 412 001
GC-RICH PCR System	100 U, 50 reactions in a final volume of 50 µl	12 140 306 001
Pwo SuperYield DNA Polymerase	100 U, 5 U/µl 40 reactions in a final volume of 50 µl	04 340 868 001
	2 x 250 U, 5 U/µl 200 reactions in a final volume of 50 µl	04 340 850 001
Water, PCR Grade	25 mL, 25 x 1 mL	03 315 932 001
	25 mL, 1 x 25 mL	03 315 959 001
	100 mL, 4 x 25 mL	03 315 843 001
Deoxynucleoside Triphosphate Set	4 x 100 µl, 4 x 10 µmol, 4 x 100 mM	11 277 049 001
	40 x 100 µl, 40 x 10 µmol, 40 x 100 mM	11 922 505 001
	4 x 250 µL, 4 x 25 µmol, 100 mM	11 969 064 001
	4 x 1,250 µL, 4 x 125 µmol, 100 mM	03 622 614 001
TriPure Isolation Reagent	50 ml	11 667 157 001
	200 ml	11 667 165 001
Expand High Fidelity^{PLUS} PCR System	500 U, 2 x 250 U, 5 U/µl 200 reactions in a final volume of 50 µl	03 300 226 001
FastStart High Fidelity PCR System	500 U, 2 x 250 U, 5 U/µl 200 reactions in a final volume of 50 µl	03 553 400 001
	2,500 U, 10 x 250 U, 5 U/µl 1,000 reactions in a final volume of 50 µl	03 553 361 001
Uracil-DNA Glycosylase, heat-labile	100 U, 1 U/µl	11 775 367 001
	500 U, 1 U/µl	11 775 375 001
Streptavidin Magnetic Particles	20 mg, 2 ml	11 641 778 001
	100 mg, 10 ml	11 641 786 001

4.4. Trademarks

EXPAND and FASTSTART are trademarks of Roche.
All other product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
Product Disclaimers.

4.6. Regulatory Disclaimer

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

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or 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.

0125.11485687001 ®

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany