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Transcriptor Reverse Transcriptase

Version: 13
Content version: October 2017

Cat. No. 03 531 287 001	2,000 U 200 reactions of 20 µl final volume 4 x 500 U
Cat. No. 03 531 295 001	500 U 50 reactions of 20 µl final volume
Cat. No. 03 531 317 001	250 U 25 reactions of 20 µl final volume

Store the enzyme at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function/ Description	Catalog Number	Content
1	red	Transcriptor Reverse Transcriptase	**Storage buffer:** 200 mM potassium phosphat, 2 mM dithiothreitol, 0.2% Triton X-100 (v/v), 50% glycerol (v/v), pH approx. 7.2	03 531 317 001	1 vial, 12.5 µl
				03 531 295 001	1 vial, 25 µl
				03 531 287 001	4 vials, 25 µl each
2	colorless	Transcriptor RT Reaction Buffer, 5x conc.	5x conc.**:** 250 mM Tris/HCl, 150 mM KCl, 40 mM $MgCl_2$, pH approx. 8.5 (25°C)	03 531 317 001	1 vial, 1 ml
				03 531 295 001	1 vial, 1 ml
				03 531 287 001	2 vials, 1 ml each

1.2. Storage and Stability

Storage Conditions (Product)

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

⚠ ***Avoid repeated freezing and thawing.***

1.3. Application

Transcriptor Reverse Transcriptase is a fast, recombinant reverse transcriptase expressed in *E. coli*. It can finalize first-strand cDNA synthesis in just 30 minutes, which reduces the total time required for RT-PCR.

- Synthesis of first strand cDNA for use in subsequent amplification reactions (RT-PCR) on thermal block cyclers or real-time instruments, such as the LightCycler® Instruments.
- RT-PCR amplification of difficult RNA templates, such as GC-rich templates with large amounts of secondary structure.
- Incorporation of Cy3-, Cy5-, DIG-, Biotin-, or aminoallyl-modified nucleotides during cDNA synthesis (*e.g.,* for use in microarray hybridization).
- Retrieval and cloning of 5’ and 3’ mRNA termini by RACE.
- Dideoxy DNA sequencing
- RNA sequencing
- 3’-end labeling of DNA fragments.
- Generation of single-stranded probes for genomic footprints.

1.4. Preparation Time

Assay Time

1st strand synthesis: 30 minutes

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Concentration of RNA Samples

If the RNA concentration of your template is low, add 10 µg/ml MS2 RNA* to stabilize the template.

Primers

The enzyme will accept three different types of primer for cDNA synthesis: Oligo(dT) primer, random hexamer primers, or a gene-specific primer.
Although the 5' ends of long mRNAs are often especially underrepresented in total mRNA, we still recommend priming with oligo(dT) for most applications. On the other hand, random hexamer primers will initiate synthesis all along the length of the RNA, permitting uniform representation of all RNA sequences in the cDNA, even those sequences that do not carry a poly(A) tail.

Concentration of Oligo(dT)$_{15}$ Primer

Add oligo(dT)$_{15}$ primer at a concentration of 1 to 10 µM. Use a 100 pmol/µl (100 µM) stock solution for high primer concentrations. To obtain low concentrations of oligo(dT)$_{15}$ primer, use an appropriate primer dilution, such as 10 pmol/µl and adjust the required volume accordingly (*e.g.*, use 2 µl of a 10 pmol/µl dilution for a final concentration of 1 µM).

Concentration of Random p(dN)$_6$ Primers

The ratio of random primers to RNA may be adjusted to control the average length of cDNA products. The high ratio recommended in this Instructions for Use will generate relatively short cDNAs, but should increase the likelihood of copying the entire target sequence (fragments up to 6 kb were amplified by PCR under the recommended conditions). If you want to synthesize longer cDNAs, you can decrease the concentration of random primers down to a minimum of 0.1 µg in the cDNA synthesis reaction.

2.2. Protocols

cDNA Synthesis

The following conditions describe synthesis of first-strand cDNA for a two-step RT-PCR.
To minimize the risk of RNase contamination, autoclave all vessels and pipette tips that will be used in the cDNA synthesis reaction. Wear gloves at all times.

1. Thaw all necessary components and place them on ice.
   – Briefly centrifuge all reagents before starting.

   Keep all reagents on ice after thawing.

2. To set up a 20 µl reaction, pipet the following components into a thin-walled RNase- and DNase-free reaction tube, on ice:

Reagent	Volume	Final conc.
Water, PCR grade*	add up to 13 µl	–
Template RNA	x µl	**Total RNA:** 1 µg (10 ng – 5 µg) **mRNA:** 10 ng (1 ng – 100 ng)
Oligo $(dT)_{15}$ primer*	y µl	1 – 10 µM
Or random primer $p(dN)_6$*, 50 A_{260} units	2 µl	0.08 A_{260} units (3.2 µg)
Or specific primer	y µl	0.5 – 2.5 µM
Final volume	**13 µl**	

3. **Optional:** Incubate at 65°C for 10 min, then place the tube immediately on ice, This step ensures denaturation of RNA secondary structures.

4. Add the following components:

Reagent	Volume	Final conc.
Transcriptor RT Reaction Buffer, 5x	4 µl	1x
Protector RNase Inhibitor* (40 U/µl)[1]	0.5 µl	20 U
dNTP-Mix*, 10 mM	2 µl	1 mM each
Transcriptor Reverse Transcriptase	0.5 µl	10 U

   – Mix well by vortexing.
   – Spin the tube briefly in a microfuge..

5. Incubate for 30 min at 55°C.

   If using random hexamer primers, reduce the incubation temperature to allow efficient annealing. Perform a two-step incubation: 10 min at 25°C, followed by 30 min at 55°C.

6. Inactivate Transcriptor Reverse Transcriptase by heating to 85°C for 5 min.
   –Place the tube on ice.

7. At this point, the reaction tube may be stored at +2 to +8°C for 1 – 2 h or at −15 to −25°C for longer time periods.

[1] Protector RNase Inhibitor is active up to 60°C.

Determination of Quality and Size

The quality and size of first strand cDNA products can be determined by gel electrophoresis on a denaturing alkaline agarose gel (Sambrook, J. & Russell, D. W., 2001).
Approximate size determinations can more easily be made on neutral agarose gels after denaturing the sample with NaOH (Lenstra, J. A., et al., 1988).

RT-PCR

The resulting single-stranded cDNA can be amplified in a polymerase chain reaction with sequence-specific primers. First-strand cDNA can be used directly in the PCR reaction, without prior purification.

- Use 1 – 5 µl (standard: 2 µl) of the cDNA reaction in the subsequent PCR (total reaction volume, 50 µl).
- Use 2 – 5 µl of the cDNA reaction or dilutions of it in a 20 µl PCR reaction in the LightCycler® Instruments*.

For reaction details and recommendations, please see the Instructions for Use for Taq DNA Polymerase*, FastStart Taq DNA Polymerase*, the Expand System*, or reagents for the LightCycler® Instruments*. FastStart Taq DNA Polymerase is recommended for quantitative RT-PCR.

Each µl of the 20 µl cDNA reaction contributes 0.4 mM $MgCl_2$ to the following PCR reaction. Optimize the $MgCl_2$ concentration of the PCR reaction if necessary.

2.3. Other Parameters

Inactivation

Transcriptor Reverse Transcriptase is inactivated by incubation at +85°C for 5 minutes.

Incorporation of Modified Nucleotides

Direct Labeling of cDNA by Incorporation of Modified Nucleotides (such as Cy3-, Cy5-, biotin-, DIG-, or aminoallyl-dUTP), to Generate a Target for Microarray Hybridization

Use single nucleotides instead of dNTP mix. Start with a 1:4 ratio of labeled to unlabeled nucleotide (1 mM labeled dUTP; 3 mM dTTP; 5 mM dATP, 5 mM dCTP, 5 mM dGTP; final reaction concentrations). Altering the ratio to 2:3 or 3:2 may help increase the signal in specific array applications (especially if the label is aminoallyl-dUTP). In addition, use a reaction temperature of +39°C to +42°C to reduce gene-to-gene variations.

Prevention of Carryover Contamination

Compatible with PCR reactions that incorporate dUTP.

Speed

30 minutes
Reverse transcription

Working Concentration

Enzyme Concentration

For RT-PCR, use 1 to 40 U Transcriptor Reverse Transcriptase per reaction, depending on the template amount. Use 10 U for 1 µg RNA template.

3. Troubleshooting

Observation	Possible cause	Recommendation
No or low yield of cDNA product	RNA template problems.	Check quality and concentration of template: ▪ Analyze an aliquot on a denaturing agarose gel to check for possible degradation. ▪ Perform a control reaction on template with an established primer pair and RT-PCR system. ▪ Check or repeat purification of template. ▪ Determine the concentration of your RNA template by measuring the A_{260} in a spectrophotometer. ▪ Use 10 ng to 5 µg of total RNA and 1 to 100 ng of mRNA. If you must use lower amounts of RNA, you may obtain better results by priming with a gene-specific primer. ▪ Dilutions of RNA samples should be done with 10 µg/ml MS2 RNA* to stabilize the RNA.
	RNase contamination	Protect RNA from ribonuclease degradation during the cDNA reaction by adding Protector RNase Inhibitor*. Inhibitor concentrations up to 60 U will not interfere with the RT-PCR.
		Use RNase-free tubes and pipette tips
	Difficult template with secondary structure (GC-rich templates).	Increase the reaction temperature up to 65°C.
		For mRNAs up to 4 kb, perform reverse transcription at 55°C for 30 min.
		Use the GC-rich Resolution Solution when working with FastStart Taq DNA Polymerase*
No or low yield of cDNA product	Enzyme concentration too high or low.	Use random hexamer primers or a gene-specific primer in the reverse transcription reaction.
		Add DMSO (up to 10%) when working with FastStart High Fidelity PCR System*
		Use PCR primers closer to the 3' terminus of the target cDNA
		Do not use more than 10 U Transcriptor Reverse Transcriptase to transcribe 1 µg total RNA template in a 20 µl cDNA synthesis reaction.
		For >1 µg total RNA, increase reaction volume and amount of Transcriptor Reverse Transcriptase proportionally.
		For low template concentrations, use less Transcriptor Reverse Transcriptase.
	Reaction temperature too high or low.	The reaction temperature should be between 42°C and 65°C. For transcripts >4kb, perform the reaction at 50°C (maximum 60°C) for 1 h. Prolonged incubation at lower temperatures will increase the yield of full-length product.
	Wrong gene-specific primer.	Try another gene-specific primer or switch to an anchored-oligo(dT) primer (*e.g.*, the anchored oligo$(dT)_{18}$ primer included in the Transcriptor First Strand cDNA Synthesis Kit*). Make sure that the gene-specific primer is able to bind to the mRNA (antisense direction).
	Inhibitors of RT reaction.	Remove inhibitors by precipitating the mRNA before first strand synthesis. Include a 70% ethanol wash step. Remove the ethanol completely.

Observation	Possible cause	Recommendation
No or low yield of PCR product *For more details, refer to the Instructions for Use of the specific PCR reagent.*	Contamination by genomic DNA.	Design primers in different exons to distinguish between potential genomic DNA contaminants and cDNA.
		Always include a control that contains no Reverse Transcriptase during the cDNA synthesis step.
	$MgCl_2$ concentration for following PCR too low or high.	Each µl of the 20 µl cDNA reaction contributes 0.4 mM $MgCl_2$ to the subsequent PCR reaction. Optimize the $MgCl_2$ concentration of the PCR reaction if necessary.
		Optimize $MgCl_2$ concentration for each template and primer combination.
	Annealing temperature too low.	Increase annealing temperature to accommodate the melting temperature of the primers used.
	Primer design for PCR not optimal.	Design alternative primers.
		Both primers should have similar melting temperatures.
	Primer concentration in PCR not optimal.	Both primers should be present at the same concentration.
		Titrate primer concentration (0.1 – 0.6 µM).
	Formation of primer-dimers.	Use FastStart Taq DNA Polymerase or FastStart High Fidelity PCR System. Design primers that do not contain complementary sequences.
		Make sure a denaturation step is included at the end of the cDNA synthesis reaction (5 min at 85°C).

4. Additional Information on this Product

4.1. Test Principle

The enzyme has RNA-directed DNA polymerase activity, DNA-dependent DNA polymerase activity, unwinding activity, and RNase H activity that degrades RNA in RNA:DNA hybrids. Thus, there is no need to perform an additional time-consuming RNase H incubation step after reverse transcription. If the RNA template is not degraded after first-strand cDNA synthesis, it can bind to the newly synthesized cDNA and make it less accessible to primers during subsequent PCR amplification. The integral RNase H activity can destroy the template, preventing this problem and improving the sensitivity of the RT-PCR analysis (Polumuri, S. K., et al., 2002). The enzyme accepts both single-stranded RNA and single-stranded DNA templates for primer-directed reverse transcription.
Transcriptor Reverse Transcriptase is recommended for RT-PCR because it is both highly sensitive and very thermostable. The enzyme is able to synthesize long cDNA products (up to 14 kb) and can be used at temperatures up to 65°C. Due to its thermostability, Transcriptor Reverse Transcriptase can transcribe GC-rich templates with large amounts of secondary structure without the help of reaction additives.

4.2. References

- Lenstra JA, de Groot RJ, Jacobs L, Kusters JG, Niesters HG, van der Zeijst BA - Synthesis of long cDNA from viral RNA template (1988) *Gene Anal Tech* **3**, 57-61
- Polumuri SK, Ruknudin A, Schulze DH - RNase H and its effects on PCR (2002) *BioTechniques* **6**, 1224-1225
- Sambrook J, Russel DW - Molecular Cloning: A Laboratory Manual (2001)

4.3. Quality Control

Each lot of Transcriptor Reverse Transcriptase is function tested in a RT-PCR reaction on a thermal block cycler (see below) and on the LightCycler® System using the LightCycler® h-PBGD Housekeeping Gene Set*. Additionally, Transcriptor Reverse Transcriptase is tested for contaminating activities as described below.

Function Tested in RT-PCR

Transcriptor Reverse Transcriptase is function tested using 2 µg of total human skeletal muscle RNA, 12.5 U Transcriptor Reverse Transcriptase, and an anchored oligo(dT)$_{18}$ primer in a volume of 20 µl. In the subsequent PCR performed with the Expand Long Template PCR System*, a 5'-CAA TCC ATG GGC AAA CTG TAT TCA CTC-3' forward primer and a 5'AGC AGG TAA GCC TGG ATG ACT GAC TAG AAG-3' reverse primer, 30 cycles of amplification generate a 10 kb fragment that is visible on an agarose gel (after it is stained with ethidium bromide).

Function Tested in Two-Step RT-PCR Using the LightCycler® Instrument

Transcriptor Reverse Transcriptase is function tested using 5 × 10^2 to 5 × 10^6 copies/5 µl of *in vitro*-transcribed human PBGD RNA. The subsequent PCR produces distinct crossing points and measurable fluorescence.

Absence of Endonuclease

1 µg LS III DNA is incubated with up to 25 U Transcriptor Reverse Transcriptase in 50 µl Transcriptor RT Buffer at 37°C for 16 h. No alteration of the banding pattern is seen.

Absence of Nicking Activity

1 µg supercoiled pBR322 DNA is incubated with up to 25 U of Transcriptor Reverse Transcriptase in 50 µl Transcriptor RT Buffer at 37°C for 16 h. No relaxation of supercoiled DNA is seen.

Absence of Ribonuclease

5 µg of MS2 RNA are incubated with up to 40 U Transcriptor Reverse Transcriptase for 4 h at 37°C in a final volume of 50 µl Transcriptor RT Buffer. No degradation of MS2 RNA is seen.

4.4. Other Parameters

Bioburden

≤50 cfu/ml

Cofactors

Mg^{2+}

Contaminants

No animal-derived additives.

Purity

≥90% (SDS-Page)

RNase H Activity

Yes

Sensitivity

When the enzyme reverse transcribes 50 pg total RNA using an oligo$(dT)_{20}$ primer, subsequent PCR amplification of 1/20 of the RT reaction generates a detectable product from a 2 kb cDNA. When the enzyme reverse transcribes 50 ng total RNA using an oligo$(dT)_{15}$ primer, subsequent PCR amplification of 1/10 of the RT reaction generates a detectable product from a 14 kb cDNA.

Specific Activity

50 U/µg

Temperature Optimum

Reaction Temperature

Perform the reaction at a temperature between +42°C and +65°C. The actual reaction temperature depends on the length of cDNA to be synthesized and the GC content of the target mRNA. For transcripts >4 kb, incubate the reaction at +50°C (maximum +60°C) for 1 h. Prolonged incubation at lower temperatures will increase the yield of full-length product.

Reaction Temperature When Using Random Hexamer Primers

Reduce the incubation temperature to allow efficient annealing. Perform a two-step incubation: 10 minutes at +25°C, followed by 30 minutes at +55°C.

Unit Definition

One unit is the enzyme activity which incorporates 1.0 nmol of [^{3}H]TMP into acid insoluble products in 10 min at 37°C with poly(A) × $(dT)_{15}$ as substrate.

Volume Activity

20 U/µl

5. Supplementary Information

5.1. 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	
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.

5.2. Changes to previous version

Editorial changes.
Layout changes.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents , kits		
Taq DNA Polymerase (1 U/µl), dNTPack	250 U, 200 reactions in a final volume of 50 µl	04 738 225 001
	1,000 U, 4 x 250 U, 800 reactions in a final volume of 50 µl	04 738 241 001
Expand Long Range dNTPack	175 U, 5 U/µl, 50 reactions in a final volume of 50 µl	04 829 034 001
	700 U, 5 U/µl, 200 reactions in a final volume of 50 µl	04 829 042 001
	3,500 U, 5x 700U, 1,000 reactions in a final volume of 50 µl	04 829 069 001
FastStart Taq DNA Polymerase, dNTPack	100 U, 5 U/µl, 50 reactions in a final volume of 50 µl	04 738 314 001
	500 U, 2 x 250 U, 250 reactions in a final volume of 50 µl	04 738 357 001
	1,000 U, 4 x 250 U, 500 reactions in a final volume of 50 µl	04 738 381 001
	2,500 U, 10 x 250 U, 1,250 reactions in a final volume of 50 µl	04 738 403 001
	5,000 U, 20 x 250 U, 2,500 reactions in a final volume of 50 µl	04 738 420 001
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
RNA, MS2	500 µl, 10 A260 units	10 165 948 001
Primer for cDNA Synthesis	40 µg	10 814 270 001
Primer “random”	2 mg, Primer, Random pd(N)6 Potassium Salt	11 034 731 001
Protector RNase Inhibitor	2,000 U, (40 U/µl)	03 335 399 001
	10,000 U, 5 x 2,000 U	03 335 402 001
Transcriptor First Strand cDNA Synthesis Kit	1 kit, 50 reactions, including 10 control reactions	04 379 012 001
	1 kit, 100 reactions	04 896 866 001
	1 kit, 200 reactions	04 897 030 001

5.4. Trademarks

EXPAND, FASTSTART and LIGHTCYCLER are trademarks of Roche.
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5.5. License Disclaimer

For patent license limitations for individual products please refer to: **List of LifeScience products**

5.6. Regulatory Disclaimer

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

5.7. Safety Data Sheet

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

5.8. 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.

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