

# Transcriptor Reverse Transcriptase

**Cat. No. 03 531 317 001** 250 U for 25 reactions  
**Cat. No. 03 531 295 001** 500 U for 50 reactions  
**Cat. No. 03 531 287 001** 2 000 U (4 × 500 U) for 200 reactions

**Version August 2007**  
 Store at -15 to -25°C

## 1. What this Product Does

### Number of Tests

The kit is designed for

- approx. 25 reactions (Cat. No. 03 531 317 001)
  - approx. 50 reactions (Cat. No. 03 531 295 001)
  - approx. 200 reactions (Cat. No. 03 531 287 001)
- with a final reaction volume of 50 µl each.

### Pack Content

Vial	Label	Content
RT red cap	Transcriptor Reverse Transcriptase (20 U/µl)	<ul style="list-style-type: none"> <li>• 12.5 µl (250 U pack size)</li> <li>• 25 µl (500 U pack size)</li> <li>• 4 × 25 µl (2,000 U pack size)</li> </ul> <p><b>Storage buffer:</b> 200 mM potassium phosphate, 2 mM dithiothreitol, 0.2% Triton X-100 (v/v), 50% glycerol (v/v), pH approx. 7.2</p>
RT buffer 5× colorless cap	Transcriptor RT Reaction Buffer (5×)	<ul style="list-style-type: none"> <li>• 1 ml (250 U pack size and 500 U pack size)</li> <li>• 2 × 1 ml (2,000 U pack size)</li> </ul> <p>5× conc.: 250 mM Tris/HCl, 150 mM KCl, 40 mM MgCl<sub>2</sub>, pH approx. 8.5 (25°C)</p>

### Storage and Stability

Store the product at -15 to -25°C through the expiration date printed on the label.

⚠ Avoid repeated freezing and thawing.

### Applications

- Synthesis of first strand cDNA for use in subsequent amplification reactions (RT-PCR) on thermal block cyclers or real-time instruments, e.g., 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

## Enzyme Properties

Unit Definition	One unit is the enzyme activity which incorporates 1.0 nmol of [ <sup>3</sup> H]TMP into acid insoluble products in 10 min at 37°C with poly(A) × (dT) <sub>15</sub> as substrate.
Volume activity	20 U/µl
Specific activity	50 U/µg
Source	New recombinant reverse transcriptase expressed in <i>E. coli</i>
Divalent ion requirement	Mg <sup>2+</sup>
Reaction Temperature	42°C – 65°C
Length of cDNA products	Up to 14 kb
RNase H activity	yes
Incorporation of modified nucleotides	Accepts labeled nucleotides like DIG-, Biotin-, Cy3-, Cy5- or aminoallyl-dUTP.
Sensitivity	When the enzyme reverse transcribes 50 pg total RNA using an oligo(dT) <sub>20</sub> 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) <sub>15</sub> primer, subsequent PCR amplification of 1/10 of the RT reaction generates a detectable product from a 14 kb cDNA.
Prevention of carry-over contamination in PCR	Compatible with PCR reactions that incorporate dUTP.
Purity (SDS-PAGE)	≥ 90%
Bioburden	≤ 50 cfu/ml
Inactivation	Transcriptor Reverse Transcriptase is inactivated by incubation at 85°C for 5 min.
Animal-derived additives	none

## 2. How to Use this Product

### Product Description

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

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 (1). 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. 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.

### Standard RT-PCR Procedure

#### Working Instructions

Please refer to the following table.

Concentration of RNA samples	If the RNA concentration of your template is low, add 10 µg/ml MS2 RNA* to stabilize the template.
Concentration of oligo(dT) <sub>15</sub> primer	Add oligo(dT) <sub>15</sub> primer at a concentration of 1-10 µM. Use a 100 pmol/µl (100 µM) stock solution for high primer concentrations. To obtain low concentrations of oligo(dT) <sub>15</sub> primer use an appropriate primer dilution, e.g., 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) <sub>6</sub> 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 manual will generate relatively short cDNAs, but should increase the likelihood of copying the complete 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.
Direct labeling of cDNA by incorporation of modified nucleotides (such as Cy3-, Cy5-, biotin-, DIG-, or aminoallyl-dUTP), to generate a target for microarray hybridizations	Use single nucleotides instead of dNTP mix. Start with a 1:4 ratio of labeled to unlabeled nucleotide (1mM labeled dUTP; 3mM dTTP; 5mM 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 since this will reduce gene-to-gene variations.
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 min at 25°C, followed by 30 min at 55°C.
Amount of Transcriptor Reverse Transcriptase per reaction	Use 1-40 U Transcriptor Reverse Transcriptase per reaction, depending on the template amount. Use 10 U for 1 µg RNA template.

### 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.

- Thaw all necessary components and place them on ice.
  - Briefly centrifuge all reagents before starting.
  - Ⓢ Keep all reagents on ice after thawing.

- 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) <sub>15</sub> primer*	y µl	1 - 10 µM
Or random primer p(dN) <sub>6</sub> *, 50 A <sub>260</sub> units	2 µl	0.08 A <sub>260</sub> units (3.2 µg)
Or specific primer	y µl	0.5 - 2.5 µM
<b>Final volume</b>	<b>13 µl</b>	

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

- Add the following components:

Reagent	Volume	Final conc.
Transcriptor RT Reaction Buffer, 5x	4 µl	1x
Protector RNase Inhibitor* (40 U/µl) <sup>1)</sup>	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.
- <sup>1)</sup> Protector RNase Inhibitor is active up to 60°C.

- 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.

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

- 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.

### 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 (2).

Approximate size determinations can more easily be made on neutral agarose gels after denaturing the sample with NaOH (3).

## 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  $\mu\text{l}$  (standard: 2  $\mu\text{l}$ ) of the cDNA reaction in the subsequent PCR (total reaction volume, 50  $\mu\text{l}$ ).
- Use 2 – 5  $\mu\text{l}$  of the cDNA reaction or dilutions of it in a 20  $\mu\text{l}$  PCR reaction in the LightCycler<sup>®</sup> Instruments.

For reaction details and recommendations, please see the instruction manuals for Taq DNA Polymerase\*, FastStart Taq DNA Polymerase\*, the Expand System\* or reagents for the LightCycler<sup>®</sup> Instruments\*. FastStart Taq DNA Polymerase is recommended for quantitative RT-PCR.

- ③ Each  $\mu\text{l}$  of the 20  $\mu\text{l}$  cDNA reaction contributes 0.4 mM  $\text{MgCl}_2$  to the following PCR reaction. Optimize the  $\text{MgCl}_2$  concentration of the PCR reaction if necessary.

## 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<sup>®</sup> System using the LightCycler<sup>®</sup> 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  $\mu\text{g}$  of total human skeletal muscle RNA, 12.5 U Transcriptor Reverse Transcriptase and an anchored oligo(dT)<sub>18</sub> primer in a volume of 20  $\mu\text{l}$ . In the subsequent PCR, performed with 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 LightCycler<sup>®</sup> Instrument

Transcriptor Reverse Transcriptase is function tested using  $5 \times 10^2$  to  $5 \times 10^6$  copies/5  $\mu\text{l}$  of *in vitro* transcribed human PBGD RNA. The subsequent PCR produces distinct crossing points and measurable fluorescence.

### Absence of Endonuclease

1  $\mu\text{g}$  LS III DNA is incubated with up to 25 U Transcriptor Reverse Transcriptase in 50  $\mu\text{l}$  Transcriptor RT Buffer at 37°C for 16 h. No alteration of the banding pattern is seen.

### Absence of "Nicking Activity"

1  $\mu\text{g}$  supercoiled pBR322 DNA is incubated with up to 25 U of Transcriptor Reverse Transcriptase in 50  $\mu\text{l}$  Transcriptor RT Buffer at 37°C for 16 h. No relaxation of supercoiled DNA is seen.

### Absence of Ribonuclease

5  $\mu\text{g}$  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  $\mu\text{l}$  Transcriptor RT Buffer. No degradation of MS2 RNA is seen.

## 4. Troubleshooting

### No or low yield of cDNA product

Possible Cause	Recommendation
RNA template problems	<p>Check quality and concentration of template:</p> <ul style="list-style-type: none"><li>• Analyze an aliquot on a denaturing agarose gel to check for possible degradation.</li><li>• Perform a control reaction on template with an established primer pair and RT-PCR system.</li><li>• Check or repeat purification of template.</li><li>• Determine the concentration of your RNA template by measuring the <math>A_{260}</math> in a spectrophotometer.</li><li>• Use 10 ng to 5 <math>\mu\text{g}</math> 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.</li><li>• Dilutions of RNA samples should be done with 10 <math>\mu\text{g}/\text{ml}</math> MS2 RNA* to stabilize the RNA.</li></ul>
RNase contamination	<ul style="list-style-type: none"><li>• 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.</li><li>• Use RNase-free tubes and pipet tips.</li></ul>
Difficult template with secondary structure (GC-rich templates)	<ul style="list-style-type: none"><li>• Increase the reaction temperature up to 65°C.</li><li>• For mRNAs up to 4 kb, perform reverse transcription at 55°C for 30 min.</li><li>• Use the GC-rich Resolution Solution when working with FastStart Taq DNA Polymerase.</li></ul>
Enzyme concentration too high or low	<ul style="list-style-type: none"><li>• Use random hexamer primers or a gene-specific primer in the reverse transcription reaction.</li><li>• Add DMSO (up to 10%) when working with FastStart High Fidelity PCR System.</li><li>• Use PCR primers closer to the 3' terminus of the target cDNA.</li><li>• Do not use more than 10 U Transcriptor Reverse Transcriptase to transcribe 1 <math>\mu\text{g}</math> total RNA template in a 20 <math>\mu\text{l}</math> cDNA synthesis reaction.</li><li>• For &gt; 1 <math>\mu\text{g}</math> total RNA increase reaction volume and amount of Transcriptor Reverse Transcriptase proportionally.</li><li>• For low template concentrations, use less Reverse Transcriptase.</li></ul>
Reaction temperature too high or low	<p>The reaction temperature should be between 42°C and 65°C. For transcripts &gt;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.</p>
Wrong gene-specific primer	<p>Try another gene-specific primer or switch to an anchored-oligo(dT) primer (e.g., the anchored oligo(dT)<sub>18</sub> 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).</p>
Inhibitors of RT reaction	<p>Remove inhibitors by precipitating the mRNA before first strand synthesis. Include a 70% ethanol wash step. Remove the ethanol completely.</p>

\* available from Roche Applied Science

## No or low yield of PCR product

For more details please see the instruction manual of the specific PCR reagent.

Possible problem	Recommendation
Contamination by genomic DNA	<ul style="list-style-type: none"> <li>Design primers in different exons to distinguish between potential genomic DNA contaminants and cDNA.</li> <li>Always include a control that contains no Reverse Transcriptase during the cDNA synthesis step.</li> </ul>
MgCl <sub>2</sub> concentration for following PCR too low or high	<ul style="list-style-type: none"> <li>Each µl of the 20 µl cDNA reaction contributes 0.4 mM MgCl<sub>2</sub> to the subsequent PCR reaction. Optimize the MgCl<sub>2</sub> concentration of the PCR reaction if necessary.</li> <li>Optimize MgCl<sub>2</sub> concentration for each template and primer combination.</li> </ul>
Annealing temperature too low	Increase annealing temperature to accommodate the melting temperature of the primers used.
Primer design for PCR not optimal	<ul style="list-style-type: none"> <li>Design alternative primers.</li> <li>Both primers should have similar melting temperatures.</li> </ul>
Primer concentration in PCR not optimal	<ul style="list-style-type: none"> <li>Both primers should be present at the same concentration.</li> <li>Titrate primer concentration (0.1 – 0.6 µM).</li> </ul>
Formation of primer dimers	<ul style="list-style-type: none"> <li>Use FastStart Taq DNA Polymerase* or FastStart High Fidelity PCR System*. Design primers that do not contain complementary sequences.</li> <li>Make sure a denaturation step is included at the end of the cDNA synthesis reaction (5 min at 85°C).</li> </ul>

## 5. References

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### 5.1 Changes to previous version

- Editorial corrections

## 6. Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our home page, [www.roche-applied-science.com](http://www.roche-applied-science.com), and our Special Interest Sites including:

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- Automated Sample Preparation (MagNA Lyser Instrument, MagNA Pure Compact System, and MagNA Pure LC System): <http://www.mag-napure.com>
- DNA & RNA preparation - Versatile tools for manual nucleic acid purification: <http://www.roche-applied-science.com/sis/napure>

Product	Pack size	Cat. No.
Transcriptor First Strand cDNA Synthesis Kit	1 kit (50 reactions)	04 379 012 001
	1 kit (100 reac.)	04 896 866 001
	1 kit (200 reac.)	04 897 030 001
Protector RNase Inhibitor	2,000 U	03 335 399 001
	10,000 U	03 335 402 001
Taq DNA Polymerase, dNTPack 1 U/µl	250 U	04 738 225 001
	4 × 250 U	04 738 241 001
Taq DNA Polymerase, dNTPack 5 U/µl	100 U	04 728 866 001
	2 × 250 U	04 728 874 001
	4 × 250 U	04 728 882 001
	10 × 250 U	04 728 904 001
	20 × 250 U	04 728 858 001
Taq DNA Polymerase, GMP Grade	1,000 U (200 ml)	03 734 927 001
	5,000 U (1,000 ml)	03 734 935 001
FastStart Taq DNA Polymerase, dNTPack 5 U/µl	100 U	04 738 314 001
	2 × 250 U	04 738 357 001
	4 × 250 U	04 738 381 001
	10 × 250 U	04 738 403 001
	20 × 250 U	04 738 420 001
FastStart High Fidelity PCR System, dNTPack	125 U	04 738 250 001
	500 U (2 × 250 U)	04 738 268 001
	2,500 U	04 738 276 001
Expand Long Range, dNTPack	175 U (50 reac.)	04 829 034 001
	700 U (200 reac.)	04 829 042 001
	3,500 U	
	(1,000 reactions)	04 829 069 001
LightCycler® FastStart DNA Master SYBR Green I	96 reactions	03 003 230 001
	480 reactions	12 239 264 001
LightCycler® FastStart DNA Master <sup>PLUS</sup> SYBR Green I	96 reactions	03 515 869 001 03
	480 reactions	515 885 001
LightCycler® FastStart DNA Master HybProbes	96 reactions	03 003 248 001
	480 reactions	12 239 272 001
LightCycler® FastStart DNA Master <sup>PLUS</sup> HybProbes	96 reactions	03 515 575 001 03
	480 reactions	515 567 001
Primer for cDNA Synthesis p(dT) <sub>10</sub>	1 A <sub>260</sub> U	10 814 261 001
Primer for cDNA Synthesis p(dT) <sub>15</sub>	1 A <sub>260</sub> U	10 814 270 001
Primer, random p(dN) <sub>6</sub>	50 A <sub>260</sub> U	11 034 731 001
RNA, MS2	10 A <sub>260</sub> units	10165 948 001

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