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

Transcriptor Universal cDNA Master

Version: 05
Content Version: November 2020

Cat. No. 05 893 151 001 1 kit
100 reactions of 20µl final volume

Store the kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Content
1	red	Transcriptor Universal Reverse Transcriptase, 20x conc.	1 vial, 100 µl
2	colorless	Transcriptor Universal Reaction Buffer, 5x conc.	1 vial, 400 µl

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, this kit is stable through the expiration date printed on the label.
⚠ ***Avoid repeated freezing and thawing.***

The kit is shipped on dry ice.

1.3. Additional Equipment and Reagent required

Additional equipment and reagents required to perform cDNA synthesis using the Transcriptor Universal cDNA Master:

Standard laboratory equipment

- Nuclease-free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Sterile reaction tubes for preparing cDNA mixes and RNA dilutions
- Standard benchtop microcentrifuge

For the cDNA synthesis

- Thermal block cycler with heated lid
- Water, PCR Grade*

1.4. Application

The Transcriptor Universal cDNA Master is designed for highly sensitive and convenient cDNA synthesis in 2-step real-time RT-PCR and is compatible with the LightCycler® Carousel-Based System, the LightCycler® 480 System, or other real-time PCR instruments.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Isolated total RNA up to 1 µg, poly $(A)^+$ RNA, or lysates from tissue culture cells, and purified RNA from all types of material.

General Considerations

Precautions

Take special precautions when working with RNA:

- Always wear gloves when working with RNA. After putting on gloves, do not touch surfaces and equipment to avoid reintroduction of RNases to decontaminated material.
- Designate a special area for RNA work only.
- Treat surfaces of benches and glassware with commercially available RNase inactivating agents. Clean benches with 100% ethanol.
- Use commercially available sterile and RNase-free disposable plasticware only.
- Purchase reagents that are free of RNases. Reserve separate reagents for RNA work only. Use DEPC-treated water for all solutions.
- Keep all reagents on ice.
- Extract RNA as quickly as possible after obtaining samples. For best results, use either fresh samples or samples that have been quickly frozen in liquid nitrogen and stored at −60°C or below.

RNA Preparation

High quality intact RNA preparations are essential for quantitative RNA analysis. 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 (Sambrook, J. et al., 1989 and Rolfs, A. et al., 1992). The Transcriptor Universal cDNA Master can be used in combination with RNA purified with both the Roche High Pure and MagNA Pure kits, and with cell lysates. The Transcriptor Universal cDNA Master was successfully tested in combination with the RealTime ready Cell Lysis Kit*. For detailed protocols, refer to the Instructions for Use of the respective products. In addition, avoid any contamination with RNases from other potential sources, such as glassware, plasticware, and reagent solutions (Sambrook, J. et al., 1989). The quality of the RNA can be checked with a bioanalyzer, or by gel electrophoresis and ethidium-bromide staining. The mRNA should appear as a smear between approximately 500 bp and 8 kb. The bulk of the mRNA should be between 1.5 and 2 kb.

Prevention of Carryover Contamination

DNA Contamination

To exclude artifacts from DNA targets, such as residual genomic DNA contaminations from RNA preparations, or contaminating DNA from previous amplifications, include appropriate positive and negative control reactions.

Safety Information

For customers in the European Economic Area

Contains SVHC: octyl/nonylphenol ethoxylates. For use in research and under controlled conditions only – acc. to Art. 56.3 and 3.23 REACH Regulation.

2.2. Protocols

cDNA Synthesis Reaction

Purified RNA as Template

Use up to 2.5 µg RNA/20 µl reaction if purified RNA is used as template for the cDNA synthesis.

⚠ ***If more than 1 µg RNA was used per 20 µl cDNA synthesis reaction, do not use more than 25% cDNA in the subsequent PCR reaction. Dilute cDNA 1:10 before adding to the PCR reactions. High amounts of RNA/cDNA may inhibit the amplification reaction or may increase the baseline in SYBR Green assays.***

RNA Lysates as Template

If RNA lysates are used as template in the cDNA synthesis reaction, the components of the lysis reaction may negatively influence the cDNA synthesis reaction.

⚠ ***Using the RealTime ready Cell Lysis Kit*, a maximum of 10% of the final cDNA synthesis reaction volume should be lysate.***

Setup of the cDNA Synthesis Reaction

1. Thaw the components listed below and place on ice. Make sure that the reaction buffer is completely dissolved. The solution should be clear.

2. Vortex briefly and centrifuge all reagents before setting up the reactions.

3. Set up the reaction components in a nuclease free reaction tube placed on ice:

Reagent	Volume	Final conc.
Water, PCR Grade	x µl	-
Transcriptor Universal Reaction Buffer (Vial 2)	4 µl	1x
Transcriptor Universal Reverse Transcriptase (Vial 1)	1 µl	1x
Template RNA	x µl	2.5 µg (down to 1 pg)[1]
Total	**20 µl**	

4. Mix the reagents and centrifuge briefly to collect the sample at the bottom of the tube.

[1] Reaction volume: If higher amounts of cDNA are required, the cDNA synthesis reaction may be scaled up to at least 100 µl without influence on the product yield.

Standard Reverse Transcription Protocol

Step	Action
Primer annealing	+25°C for 5 min[1]
Reverse transcription	+55°C for 10 min[2]
Denaturation	+85°C for 5 min
Hold	+4°C (unlimited)

[1] Primer annealing: In combination with heat-labile DNAse, use +29°C for 10 min.

[2] Reverse transcription: Depending on the target used, the optimal temperature and incubation time for the reverse transcription step may vary. The enzyme can be used at temperatures between +40°C and +60°C. When establishing a new assay, it is recommended to start with +55°C for 10 min. If a higher stringency in primer annealing is required or if difficult targets (*e.g.*, GC-rich templates or templates with a high degree of secondary structures) are reverse transcribed, the temperature can be raised up to +60°C.

For PCR

The cDNA can be added to the PCR without purification.

- For PCR on one of the LightCycler® Instruments, use 2 to 5 µl of the cDNA reaction or dilutions in a 20 µl reaction.
- For initial experiments, use 2 µl cDNA template for a 20 µl PCR.

⚠ ***If more than 1 µg RNA was used per 20 µl cDNA synthesis reaction, do not use more than 25% cDNA in the subsequent PCR reaction. Dilute cDNA 1:10 before it is added to the PCR reaction. High amounts of RNA/cDNA may inhibit the amplification reaction or may increase the baseline in SYBR Green assays.***

ⓘ *Transcriptor Universal Reverse Transcriptase has RNase H activity. RNase H removes the RNA template after cDNA synthesis, allowing PCR primers to more easily bind the cDNA, which in some cases increases the sensitivity of the PCR. No separate RNase H digestion step is required.*

3. Results

Two microliter of cDNA was amplified with the LightCycler® 480 SYBR Green I Master in a LightCycler® 480 Instrument following the standard protocol.

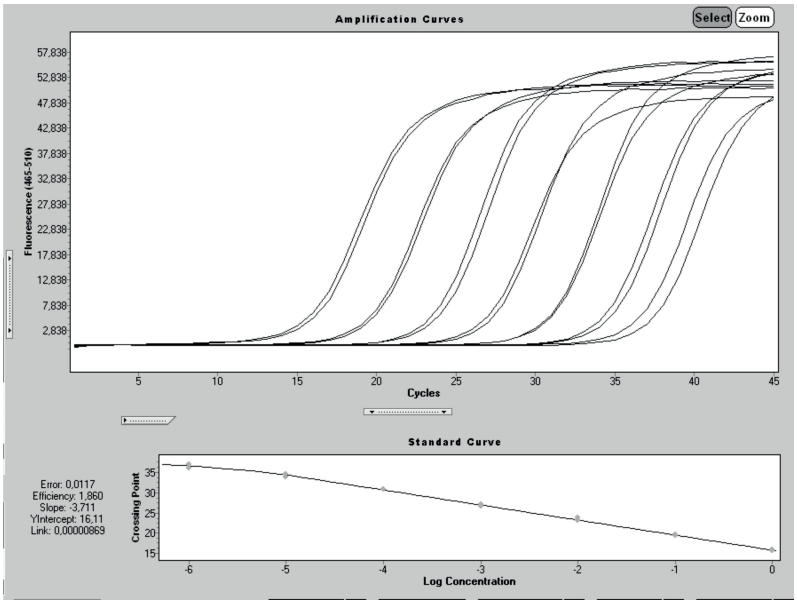

Fig. 1: 1 µg to 1 pg of total RNA from K-562 cells was reverse transcribed with Transcriptor Universal cDNA Master.

4. Troubleshooting

Observation	Possible cause	Recommendation
No PCR product or very little amount of PCR product	Insufficient amount of template RNA.	Check quality and concentration of template.
		Increase amount of RNA template in cDNA reaction (maximum 2.5 µg/20 µl reaction).
		Add 10 µg/ml MS2 RNA* to template to stabilize low concentrations of target RNA.
	Template RNA degraded.	Prepare fresh RNA template, being careful to prevent contamination with RNases.
		Check RNA preparation by gel electrophoresis.
		Add Protector RNase Inhibitor* to the cDNA synthesis step.
	Too much template RNA.	A too high amount of template RNA may affect/inhibit performance of RT-PCR; decrease amount of RNA template.
	Template secondary structure prevented effective first strand cDNA synthesis.	Raise temperature for reverse transcription reaction up to +60°C.
	Template secondary structure inhibits effective formation of full-length products.	If GC content of RNA is high (>60%), increase incubation temperature for cDNA synthesis up to 60°C, or denaturation time in the subsequent PCR.
Nonspecific PCR products	Annealing temperature too low.	Increase annealing temperature during PCR to increase specificity of amplification.
	Contaminating DNA in sample.	Perform a control without reverse transcription step; for details, see section on “DNA contamination”.
		Design primers that anneal to sequence in exons on both sides of an intron or at the exon/exon boundary of the mRNA to differentiate between amplified cDNA and potential contaminating DNA.
	Inhibitors of RT reaction.	Remove inhibitors by precipitating the mRNA, washing the precipitate with 70% ethanol, and then redissolving the precipitate.
Baseline in real-time PCR too high	Too much cDNA template in PCR reaction.	Dilute cDNA before use in real-time PCR.

5. Additional Information on this Product

5.1. Test Principle

The Transcriptor Universal cDNA Master provides a convenient solution for time-saving cDNA synthesis for use in two-step, real-time RT-PCR. All reagents needed for cDNA synthesis, including random hexamer primers, nucleotides, buffers, and enzymes are supplied in only two vials, minimizing pipetting efforts. The unique enzyme included in this kit has a broad temperature range and is suitable for high temperature reverse transcription. The amount of random hexamer primers included in the Transcriptor Universal Reaction Buffer enables high cDNA yields from all regions of the RNA template.
The product is tested for purified cellular RNA, mRNA, and cell lysates generated using the RealTime ready Cell Lysis Kit* which further accelerates fast real-time PCR directly on cell lysates.
If large amounts of cDNA are required, the reaction can be upscaled without influencing the product yield.
The Transcriptor Universal cDNA Master enables reliable cDNA synthesis over a wide dynamic range even for GC-rich templates, and is ideal for high-throughput quantitative RT-PCR analysis.

5.2. Quality Control

Each lot of the Transcriptor Universal cDNA Master is function tested in RT-PCR. Reverse transcription is performed on a dilution series of K-562 total RNA. 2 µl of cDNA is amplified with the LightCycler® 480 SYBR Green I Master* and primers specific for GAPDH in a LightCycler® 480 Instrument using the standard protocol.

6. Supplementary Information

6.1. 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.

6.2. Changes to previous version

Layout changes.
Editorial changes.
New information added related to the REACH Annex XIV.

6.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
High Pure RNA Paraffin Kit	1 kit, up to 100 isolations	03 270 289 001
mRNA Isolation Kit	1 kit	11 741 985 001
MagNA Pure Compact RNA Isolation Kit	1 kit, 4 x 8 sealed cartridges, 32 isolations	04 802 993 001
High Pure RNA Isolation Kit	1 kit, 50 isolations	11 828 665 001
High Pure RNA Tissue Kit	1 kit, 50 isolations	12 033 674 001
LightCycler® 480 SYBR Green I Master	5 x 1 ml, 2x conc., 5 x 100 reactions of 20 µl final volume each	04 707 516 001
	10 x 5 ml, 2x conc., 10 x 500 reactions of 20 µl final volume each	04 887 352 001
MagNA Pure LC RNA Isolation Kit III (Tissue)	1 kit, up to 192 isolations	03 330 591 001
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 A_{260} units	10 165 948 001
RealTime ready Cell Lysis Kit	1 kit, 50 lysis reactions with a final reaction volume of 40 µl each, 50 reactions of 40 µl final volume each	06 366 821 001
	1 kit, 500 lysis reactions with a final reaction volume of 40 µl each, 500 reactions of 40 µl final volume each	05 943 523 001
MagNA Pure LC RNA Isolation Kit - High Performance	1 kit, up to 192 isolations	03 542 394 001
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

6.4. Trademarks

MAGNA PURE and LIGHTCYCLER are trademarks of Roche.
SYBR is a trademark of Thermo Fisher Scientific Inc..
All other product names and trademarks are the property of their respective owners.

6.5. License Disclaimer

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

6.6. Regulatory Disclaimer

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

6.7. Safety Data Sheet

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

6.8. 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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