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

Transcriptor One-Step RT-PCR Kit

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Hot start one step RT-PCR kit for endpoint analysis up to 6.5 kb

Cat. No. 04 655 877 001	1 kit 50 reactions, including 10 control reactions
Cat. No. 04 655 885 001	1 kit 150 reactions

Store the kit at −15 to −25°C.

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1. General Information

1.1. Contents

Vial / Bottle	Label	Function	Catalog Number	Content
1	Enzyme Mix	Contains Transcriptor Reverse Transcriptase, Expand System, and Protector RNase Inhibitor.	04 655 877 001	1 vial, 50 µl
			04 655 885 001	3 vials, 50 µl each
2	Reaction Buffer, 5x conc.	Includes Tris, $MgCl_2$, sodium salts of dNTPs (1.5 mM each), and additives for hot start PCR.	04 655 877 001	1 vial, 500 µl
			04 655 885 001	3 vials, 500 µl each
3	Water, PCR Grade	To adjust the final reaction volume.	04 655 877 001	2 vials, 1 ml each
			04 655 885 001	6 vials, 1 ml each
4	Control RNA	HAV RNA, *in vitro* transcript.	04 655 877 001	50 µl, 200 copies/µl
5	Control Primer Mix, HAV RNA	HAV RNA upstream and downstream primer to amplify a 246 bp fragment.	04 655 877 001	20 µl, each primer 10 µM

1.2. Storage and Stability

Storage Conditions (Product)

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

Vial / Bottle	Label	Storage
1	Enzyme Mix	−15 to −25°C
2	Reaction Buffer, 5x conc.	
3	Water, PCR Grade	
4	Control RNA	−60°C or below
5	Control Primer Mix, HAV RNA	−15 to −25°C

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

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

For the RT-PCR reaction

- Standard block cycler instrument
- PCR primers
- Template RNA
- PCR reaction vessels (thin-walled PCR tubes or plates are recommended)

1.4. Application

The Transcriptor One-Step RT-PCR Kit is designed for fast, sensitive, and specific endpoint RT-PCR analysis using gene-specific primers. It combines the high sensitivity and yield of Transcriptor Reverse Transcriptase with the fidelity and yield of the Expand System. It adds high specificity and overall improved performance, such as reduced primer-dimer formation by the innovative hot start buffer. It allows reaction temperatures up to 60°C and achieves full-length transcripts up to 6.5 kb.
The kit may be applied to:

- Qualitative, semi quantitative, or quantitative analysis of RNA transcription levels even in single cells in combination with gel-based detection methods or ELISA-based detection methods.
- Cloning of RNA up to 6.5 kb.
- Mutation analysis at RNA level in combination with sequencing or other mutation.
- Scanning techniques

1.5. Preparation Time

Assay Time

1 to 2 hours for RT-PCR of a 246 bp fragment (*e.g.*, control reaction supplied with the kit).

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Isolated total RNA from 1 fg up to 1 µg, poly (A)+ RNA, *in vitro*-transcribed RNA, or lysates from cell culture.

Primers

Primer Design

Sequence-specific primers must be used for one-step RT-PCR. To differentiate between amplification of cDNA and amplification of contaminating genomic DNA, primers can be designed that anneal to sequences in exons on both sides of an intron. With this approach, PCR products from genomic DNA will be much longer compared to the intronless mRNA-derived products. Alternatively, a primer designed on an exon/exon boundary of the mRNA should not amplify genomic DNA.

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

For high quality eukaryotic RNA preparations, it is necessary to minimize the activity of RNases released during cell lysis using inhibitors of RNases, or methods that disrupt cells and simultaneously inactivate RNases (Sambrook, J. et al.,1989 and Rolfs, A. et al., 1992). This kit can be used in combination with RNA purified with both the Roche High Pure and MagNA Pure kits, and with cell lysates.
Avoid contamination with RNases from other potential sources, such as glassware, plasticware, and reagent solutions (Sambrook, J. et al.,1989).

The enzyme mix (Vial 1) contains the Protector RNase Inhibitor, which inhibits a wide spectrum of RNases and is active up to +60°C. Protector RNase Inhibitor protects the RNA during cDNA synthesis, where other RNase inhibitors fail. Integrity of mRNA is particularly important when longer fragments are analyzed.

The size of the mRNA can be determined by gel electrophoresis and ethidium bromide staining. The mRNA should appear as a smear between approx. 500 bp and 8 kb. The bulk of the mRNA should be between 1.5 and 2 kb.

Prevention of Carryover Contamination

DNA Contamination

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

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

Setup of the RT-PCR Reaction

1. Thaw the components listed below and place them on ice.

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

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

Reagent	Volume	Final conc.
Water, PCR Grade (Vial 3)	× µl	
5x Reaction Buffer (Vial 2)	10 µl	1x
Fwd primer	× µl	0.4 µM
Rev primer	× µl	0.4 µM
Transcriptor Enzyme Mix (Vial 1)	1 µl	
Template RNA	× µl	100 ng (down to 1 fg)
Total	**50 µl**	

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

RT-PCR Protocol

RT-PCR is performed in a standard block cycler instrument. Depending on the instrument used, the recommended program may require optimization.
The cycle number is dependent on the abundance of the respective mRNA. For rare mRNA messages, 40 cycles or a second nested PCR could be necessary.

Reverse Transcription

Reverse transcription may be performed between 45°C and 60°C. When establishing a new assay, it is recommended to start with 50°C for 30 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 higher reaction temperatures, primers with appropriate melting temperatures must be used.

PCR

During the initial denaturation step, Transcriptor Reverse Transcriptase is inactivated and the RNA/cDNA hybrid is denatured. The recommended denaturation temperature is 94°C. A high GC content (>60%) of the template might require either a higher denaturation temperature or a longer denaturation time. The annealing temperature in PCR depends on the melting temperature of the respective primer pair. Use an appropriate computer program to calculate the optimal temperature for your primers. The recommended annealing temperature is the melting temperature of the primers or 2°C below. The elongation temperature is always 68°C. The elongation time depends on the template length: 60 seconds per kb. After 10 cycles, it is recommended to extend the elongation time by 5 seconds per kb for each successive cycle. This may result in higher yield and higher sensitivity.

Standard RT-PCR Protocol

Step	Action			
Sample loading	Overlay the reaction with 30 µl mineral oil, if required by the type of block cycler used.			
Reverse transcription	50°C for 10 - 30 min			
Initial denaturation	94°C for 7 min			
Standard PCR Profile	**Setup**	**Temp.**	**Time (s)**	**Cycles**
	Denaturation	94°C	10	10
	Annealing	×[1)]°C	30	
	Elongation	68°C	×[2])	
	Denaturation	94°C	10	25
	Annealing	×[1)]°C	30	
	Elongation	68°C	×[2)] + ×[3)]	
	Final Elong.	68°C	7 min	
	Analyze samples	1 to 3% agarose gel.		

Short RT-PCR protocol[4)]

In most cases, the short RT-PCR protocol is sufficient. Due to the shortened reaction time, yield could be reduced. Therefore, use the standard protocol if highest sensitivity is necessary.

Step	Action			
Sample loading	Overlay the reaction with 30 µl mineral oil, if required by the type of block cycler used.			
Reverse transcription	50°C for 5 min.			
Initial denaturation	94°C for 5 min			
Standard PCR Profile	**Setup**	**Temp.**	**Time (s)**	**Cycles**
	Denaturation	94°C	10	35
	Annealing	×[1)]°C	30	
	Elongation	68°C	×[2)]	
	Final Elong.	68°C	5 min	
	Analyze samples	1 to 3% agarose gel.		

1. Melting temperatures of primers or up to 2°C below.
2. Elongation time: 60 s per kb.
3. Cycle elongation of 5 s per kb for each successive cycle. Cycle elongation may result in higher yield and specificity.
4. Evaluated by using a fast cycling instrument (*e.g.,* 2720 Thermal Cycler, Applied Biosystems).

Setup of the Control Reaction

1. Thaw the components listed below and place them on ice.

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

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

Reagent	Volume	Final conc.
Water, PCR Grade (Vial 3)	32 µl	
5x Reaction Buffer (Vial 2)	10 µl	1x
Control Primer Mix (Vial 5)	2 µl	0.4 µM
Transcriptor Enzyme Mix (Vial 1)	1 µl	
Control RNA (Vial 4)	5 µl	1,000 copies
Total	**50 µl**	

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

RT-PCR Performed on a Dilution Series of HAV RNA, *In Vitro* Transcript

The Hav RNA *in vitro* transcript is provided with Cat. No. 04 655 877 001.

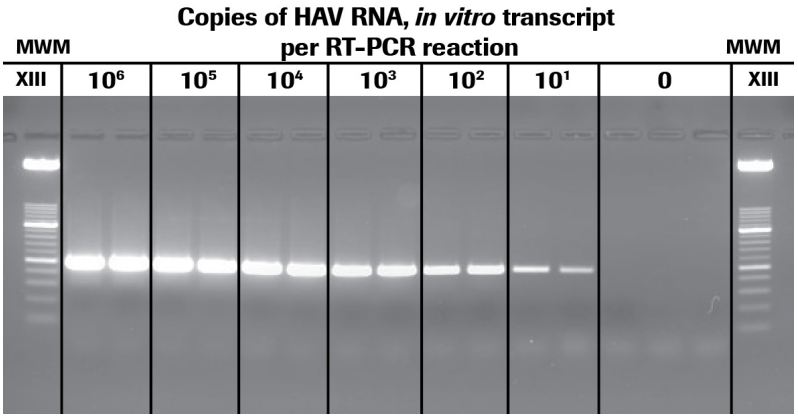

Fig. 1: RT-PCR was performed on a dilution series of the HAV RNA, in vitro transcript provided with Cat. No. 04 655 877 001. The setup of the control reaction and the subsequent RT-PCR with primers for a 246 bp HAV fragment was performed according to the standard RT-PCR protocol (reverse transcription at 50°C for 30 min, PCR annealing at 55°C).

2.3. Parameters

Maximum Fragment Size

Up to 6.5 kb

Temperature Optimum

+45 to +60°C
When establishing a new assay, for the reverse transcription step, it is recommended to start with 50°C for 30 min.

3. Results

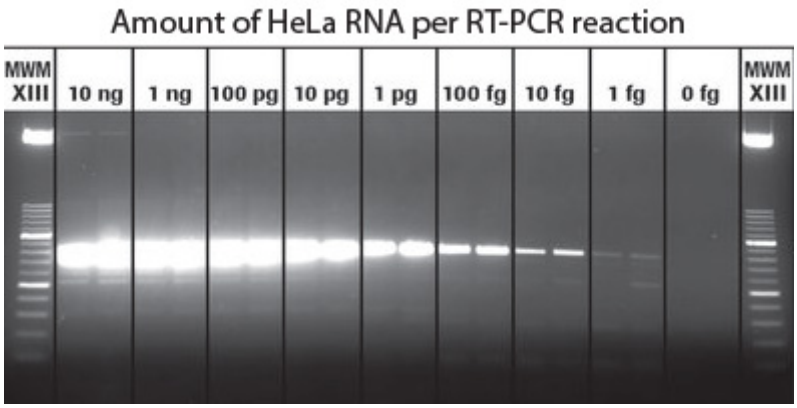

Fig. 2: High sensitivity for difficult templates. RT-PCR was performed on a dilution series of the HeLa RNA. A 389 bp fragment was amplified with primers specific for human 28S ribosomal RNA according to the standard RT-PCR protocol (reverse transcription at 50°C for 30 min). The GC content of the amplified fragment is 64%. With 1 fg of RNA, a clearly visible band is obtained after agarose gel electrophoresis and ethidium bromide staining.

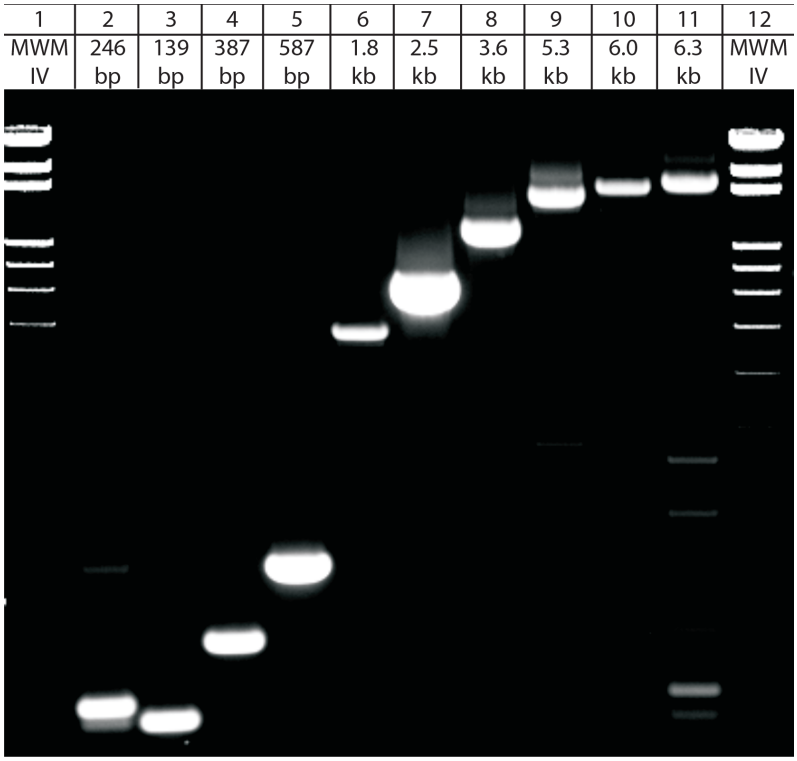

Fig. 3: Long fragments from a variety of templates. The Transcriptor One-Step RT-PCR Kit generates fragments up to 6.5 kb. RT-PCR on various fragments from different sources was performed according to the standard RT-PCR protocol. Aliquots of 15 µl of each RT-PCR reaction were analyzed on an agarose gel.

Lanes	Content
1 and12	Molecular Weight Marker IV
2	HAV (10^6 copies of HAV)
3	Apo E (10 ng of human liver RNA)
4	β-Actin (10 ng of K-562 RNA)
5	β-Actin (10 ng of K-562 RNA)
6	Dystrophin (10 ng human skeletal muscle RNA)
7	Apo B (100 ng human liver RNA)
8	Dystrophin (100 ng human skeletal muscle RNA)
9	hTSF (100 ng HeLa RNA)
10	Dystrophin (100 ng human skeletal muscle RNA)
11	mfas (100 ng mouse liver RNA)

4. Troubleshooting

Observation	Possible cause	Recommendation
No PCR product or very little amount of PCR product	Insufficient amount of template RNA.	Increase amount of RNA template in cDNA reaction.
		Use poly(A)+ mRNA rather than total RNA as template.
		Raise enzyme amount to 1.5 to 2-fold.
	Template RNA degraded.	Prepare fresh RNA template, being careful to prevent RNase activity.
		Check RNA preparation by gel electrophoresis.
	Too much template RNA.	A too high amount of template RNA may affect/inhibit performance of RT-PCR; decrease amount of RNA template.
	RT-PCR Inhibitors are present in the RNA.	Make sure that the RNA is free of RT-PCR inhibitors, such as by using Roche High Pure or MagNA Pure Kits for RNA purification and isolation.
	Reaction not optimized.	Increase primer concentration (up to 1 µM maximum).
		Synthesize the cDNA at a higher temperature.
	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 denaturation temperature or denaturation time in PCR cycles.
	Incubation temperature too high.	For higher reverse transcription reaction temperatures, primers with appropriate melting temperatures must be used. The annealing temperature in PCR depends on the melting temperature of the respective primer pair. Use an appropriate computer program to calculate the optimal temperature for the primers used. The recommended annealing temperature is the melting temperature of the primers or 2°C below.
Background smear	Secondary amplification product(s).	Check reagent concentrations and cycling conditions:
		Optimize temperature of cDNA synthesis step.
		Optimize primer concentration.
		Decrease number of cycles.
		Check and perhaps decrease concentration of template.
Nonspecific product bands	Annealing temperature too low.	Increase annealing temperature during PCR to increase specificity of amplification.
	Primer-dimers formed.	Design primers without complementary sequences at the 3' ends.
	Contaminating DNA in sample.	Perform a control without reverse transcription step.
		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.

5. Additional Information on this Product

5.1. Test Principle

The Transcriptor One-Step RT-PCR Enzyme Mix contains a blend of active components: Transcriptor Reverse Transcriptase, Protector RNase Inhibitor, and a DNA polymerase enzyme blend, consisting of Taq DNA Polymerase and a proofreading polymerase. Transcriptor Reverse Transcriptase enables sensitive and robust reverse transcription. Protector RNase Inhibitor is fully active at elevated temperatures to provide maximum template protection during reverse transcription. For the PCR step, an optimized enzyme blend is included to assure high fidelity and minimize the probability of introduction of mutations.
An optimized RT-PCR buffer, including dNTPs and additives, ensures ease of use and improved performance of a hot start system. Proprietary hot start component binds and sequesters primers at lower temperatures to prevent the primers from nonspecific binding. This new formulation is effective during reverse transcription as well as during PCR. It results in increased specificity and sensitivity. The buffer is also suited for one-step RT-PCR of difficult templates with high secondary structure and high-GC content, without an increase in the reaction temperature.

5.2. Quality Control

Each lot of the Transcriptor One-Step RT-PCR Kit is function tested in RT-PCR. RT-PCR is performed using 1,000 copies of HAV RNA, *in vitro* transcript (provided with Cat. No. 04 655 877 001). The setup of the control reaction and subsequent RT-PCR with primers for a 246 bp HAV RNA fragment is performed according to the standard RT-PCR protocol (reverse transcription at +50°C for 30 minutes), as described in the Instructions for Use. With 1,000 copies of target RNA, a clearly visible band is obtained after agarose gel electrophoresis and ethidium bromide staining.
In addition, RT-PCR is performed on a dilution series of human liver total RNA (10 ng, 1 ng, and 0.1 ng). RT-PCR with specific primers for a 2.5 kb fragment of ApoB is performed according to the standard RT-PCR protocol (reverse transcription at +50°C for 30 minutes), as described in the Intructions for Use. With 1 ng of RNA, a clearly visible band is obtained after agarose gel electrophoresis and ethidium bromide staining.

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

New information added related to the REACH Annex X.

6.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
High Pure Viral RNA Kit	1 kit, up to 100 purifications	11 858 882 001
Expand Long Range dNTPack	175 U, 1 x 175 U, 50 reactions in a final volume of 50 µl	04 829 034 001
	700 U, 1 x 700 U, 200 reactions in a final volume of 50 µl	04 829 042 001
	3,500 U, 5 x 700 U, 1,000 reactions in a final volume of 50 µl	04 829 069 001
High Pure RNA Isolation Kit	1 kit, 50 isolations	11 828 665 001
Expand High Fidelity PCR System, dNTPack	100 U, 1 x 100 U, 40 reactions in a final volume of 50 µl	04 738 250 001
	500 U, 2 x 250 U, 200 reactions in a final volume of 50 µl	04 738 268 001
	2,500 U, 10 x 250 U, 1,000 reactions in a final volume of 50 µl	04 738 276 001
mRNA Isolation Kit	1 kit	11 741 985 001
High Pure RNA Tissue Kit	1 kit, 50 isolations	12 033 674 001
High Pure RNA Paraffin Kit	1 kit, up to 100 isolations	03 270 289 001

6.4. Trademarks

MAGNA PURE and EXPAND are trademarks of Roche.
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 LifeScience 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 to display country-specific contact information.

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