For general laboratory use.

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EvoScript Universal cDNA Master

Version: 04
Content version: June 2017

Easy-to-use reaction mix for cDNA synthesis for two-step RT-qPCR

Cat. No. 07 912 374 001	1 kit up to 50 reactions of 20 µl final volume each
Cat. No. 07 912 439 001	1 kit up to 100 reactions of 20 µl final volume each
Cat. No. 07 912 455 001	1 kit up to 200 reactions of 20 µl final volume each

Store the kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Catalog Number	Content
1	blue	EvoScript Universal cDNA Master, Enzyme Mix	Contains Enzyme Blend for reverse transcription and Protector RNase Inhibitor, 10x conc.	07 912 374 001	1 vial, 100 µl
				07 912 439 001	2 vials, 100 µl each
				07 912 455 001	4 vials, 100 µl each
2	red	EvoScript Universal cDNA Master, Reaction Buffer	Contains random primers, anchored oligo(dT)$_{18}$, dNTP, and Mg(OAc)$_2$, 5x conc.	07 912 374 001	1 vial, 200 µl
				07 912 439 001	2 vials, 200 µl each
				07 912 455 001	4 vials, 200 µl each
3	colorless	EvoScript Universal cDNA Master, Water, PCR Grade	To adjust the final reaction volume.	07 912 374 001	1 vial, 1 ml
				07 912 439 001	2 vials, 1 ml each
				07 912 455 001	4 vials, 1 ml each

1.2. Storage and Stability

Storage Conditions (Product)

The kit is shipped on dry ice.
When stored at −15 to −25°C, the kit is stable through the expiration date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	blue	Enzyme Mix	Store the kit at −15 to −25°C or store at +2 to +8°C for a maximum of 4 weeks.
2	red	Reaction Buffer	
3	colorless	Water, PCR Grade	

1.3. Additional Equipment and Reagents Required

Standard Laboratory Equipment

- Nuclease-free pipette tips
- 1.5 ml RNase-free microcentrifuge tubes to prepare master mixes and dilutions.

For the cDNA Synthesis

- Thermal block cycler with heated lid

For qPCR (optional)

- Real-Time PCR systems such as the LightCycler® 480 or LightCycler® 96 Instrument*
- LightCycler® 480 Multiwell Plate 96* or 384*
- LightCycler® 8-Tube Strip Adapter Plate*
- LightCycler® 8-Tube Strips*
- Standard swinging-bucket centrifuge with rotor for multiwell plates

For qPCR Primer and Probe Design (optional)

- Universal ProbeLibrary Assay Design Center at: www.universalprobelibrary.com

For RNA Purification

- MagNA Pure 96 Instrument* including consumables
- MagNA Pure 96 DNA and Viral NA Kit, Large Volume* or
- MagNA Pure 96 DNA and Viral NA Kit, Small Volume*

Alternatively, use a different MagNA Pure System together with a dedicated reagent kit (for automated isolation), or a High Pure Nucleic Acid Isolation Kit (for manual isolation).

1.4. Application

The EvoScript Universal cDNA Master is designed for highly sensitive and convenient cDNA synthesis for use in two-step RT-qPCR and is compatible with the LightCycler® 480 System, LightCycler® 96 System, or other real-time PCR instruments. The 2-vial composition is ideally suited for reverse transcription, requiring only the addition of target RNA.

1.5. Preparation Time

Assay Time

Step	Time [min]
RT reaction setup time	30
RT reaction	35
PCR reaction setup time	30
PCR reaction	60
Total Time	2 hours 35 minutes

Typical run time

When using the recommended protocol for the reverse transcription, the EvoScript Universal cDNA Master run time is approximately 35 minutes.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Use any template RNA such as isolated total RNA, mRNA, viral RNA, or *in vitro*-transcribed RNA. Use 1 µg total RNA or 10 ng poly(A)$^+$ RNA.

⚠ ***High quality intact RNA, free of residual genomic DNA, RNase, and inhibitors is essential for good results.***

For reproducible isolation of nucleic acids, we recommend:
- Use either RNase inhibitors such as Protector RNase Inhibitor or isolation conditions that inactivate RNases.
- If necessary, analyze different steps in the process (lysis, isolation) by gel electrophoresis (ethidium bromide staining) to ensure that the sample is still RNase-free.

ⓘ *RNases can also be present on contaminated glassware.*
ⓘ *For details, see the Roche Life Science homepage, www.lifescience.roche.com.*

Control Reactions

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

General Considerations

Precautions

Use RNase-free techniques. Nuclease-contaminated reagents and reaction vessels will degrade template RNA. Please follow these guidelines to minimize risk of contamination:
- Wear disposable gloves and change them frequently.
- Avoid touching surfaces or materials that could cause nuclease carryover.
- Use only reagents provided in this kit. Substitutions may introduce nucleases.
- Clean and decontaminate work areas and instruments, including pipettes, with commercially available decontamination reagents.
- Use only new RNase-free aerosol-blocking pipette tips and siliconized microcentrifuge reaction tubes.
- Use a work area specifically designated for nucleic acid work, and if possible, use reaction vessels and pipettes dedicated only for work with template RNA.

2.2. Protocols

Standard Protocols for the cDNA Synthesis

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 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 Buffer*, a maximum of 10% of the final cDNA synthesis reaction volume should be lysate.***

Setup of the cDNA Synthesis

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

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

   ⚠ ***The Reaction Buffer, 5x conc. may appear cloudy after initial thawing. Vortex the tube containing Reaction Buffer several times and centrifuge briefly for several seconds. Make sure that the Reaction Buffer is completely dissolved and clear. If the solution is not clear, repeat the vortex mixing and centrifugation step until a clear solution is obtained.***

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

Reagent	Volume [µl]	Final conc.
Water, PCR Grade (Vial 3)	x	–
Reaction Buffer, 5x conc. (Vial 2)	4.0	1x
Template RNA	x	2.5 µg (down to 1 pg)[1]
Total Volume	**18.0**	

4. Mix the reagents and centrifuge briefly to collect the sample at the bottom of the tube.
   – Incubate the tube on ice for at least 5 minutes to let primers anneal to RNA.

5. Add 2 µl of Enzyme Mix, 10x conc. (Vial 1) to a final concentration of 1x and follow the protocol below.

   ⚠ ***We recommend adding the Enzyme Mix last.***

[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

The reverse transcription protocol shown below is optimized for the Enzyme Mix:

1. Heat to +42°C for 15 minutes.

2. Heat to +85°C for 5 minutes.

3. Heat to +65°C for 15 minutes.

4. Cool to +4°C with an unlimited **Hold** time.

5. Stop the reaction by placing the tube on ice.
   – At this point, the reaction tube may be stored at +2 to +8°C for 1 to 2 hours or at −15 to −25°C for longer periods.

PCR Reaction Protocol

The resulting 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 of it in a 20 µl reaction. For initial experiments, use 2 µl cDNA template for a 20 µl PCR.

The Enzyme Mix (Vial 1) 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.

Protocol for Use with the LightCycler® 480 Instrument II (Multiwell Plate 96 or 384)

Any real-time PCR systems such as the LightCycler® 480 Instrument* or LightCycler® 96 Instrument* can be used. For example, use the optimized protocol shown below with the LightCycler® 480 Instrument II running the qPCR with the LightCycler® Multiplex DNA Master*, primers for PCR, and hydrolysis probes.

LightCycler® Multiplex DNA Master, primers, and hydrolysis probes are not provided with this kit.*

⚠ ***Program the LightCycler® Instrument before preparing the reaction mixes.***

A LightCycler® Instrument protocol that uses the LightCycler® Multiplex DNA Master contains the following programs:

- **Pre-incubation**
- **Amplification** of the cDNA
- **Cooling** of the thermal block

For details on how to program the experimental protocol, see the current LightCycler® 480 Instrument Operator's Manual.

The following table shows the parameters that must be programmed for a real-time qPCR run using the LightCycler® Multiplex DNA Master on the LightCycler® 480 Instrument II (Multiwell Plate 96 or 384) with hydrolysis probes.

Setup					
Block Type			Reaction Volume [µl]		
96 (384)			20 (10)		
Detection Format		Excitation Filter		Emission Filter	
Mono Color Hydrolysis Probe / UPL Probe					
FAM		465		510	
Programs					
Program Name		Cycles		Analysis Mode	
Pre-Incubation		1		None	
Amplification		45[1]		Quantification	
Cooling		1		None	
Temperature Targets					
	Target [°C]	Acquisition Mode	Hold [hh:mm:ss]	Ramp Rate [°C/s]	Acquisitions [per °C]
Pre-Incubation	95	None	00:00:30	4.4 (4.8)	–
Amplification	95	None	00:00:05	4.4 (4.8)	–
	60	Single	00:00:30	2.2 (2.5)	–
Cooling	40	None	00:00:30	2.2 (2.5)	–

[1] Forty-five cycles is suitable for most assays. If the assay shows steep amplification curves and early crossing points, 40 cycles should be sufficient. Reducing the number of cycles will improve uniformity of the product and reduce the time required for the assay.

Setup of the PCR Reaction for the LightCycler® 480 Instrument II

Follow the procedure below to prepare at least ten 20 µl standard reactions. 10 µl amplifications included in parenthesis to be used for 384-well plate setups.

For more information, refer to the Instructions for Use of the LightCycler® Multiplex DNA Master.

⚠ ***Do not touch the surface of the LightCycler® 480 Multiwell Plate/LightCycler® 8-Tube Strips.***

1. Thaw the solutions and, to ensure recovery of all the contents, briefly spin vials in a microcentrifuge before opening.
   – Mix carefully by pipetting up and down or vortex briefly. Place samples on ice.

2. Prepare a 20x conc. solution of your primers and a 20x conc. solution of your probes.

3. In a 1.5 ml reaction tube, prepare the qPCR Mix and put on ice.
   – For best results, prepare at least 10 reactions in order to reduce pipetting errors. To prepare more reactions, multiply the amount in the “Volume 1 Reaction” column below by the number of reactions to be run, plus at least one additional reaction.

Reagent	Volume 1 Reaction [µl]	Volume 10 Reactions [µl]	Final conc.
Water, PCR Grade (Vial 3)	12.0 (6.0)	120.0	
qPCR Reaction Mix	4.0 (2.0)	40.0	1x
Primer Mix, 20x	1.0 (0.5)	10.0 (5.0)	1x
Probe Mix, 20x	1.0 (0.5)	10.0 (5.0)	1x
Total Volume	**18.0 (9.0)**	**180.0 (90)**	

4 Mix carefully by pipetting up and down or vortex briefly. Place on ice.

Although we recommend working on ice and preparing the reagents right before use, the working solution (everything combined except cDNA template) is stable at +15 to +25°C, for up to 4 hours, and is therefore ideal for use in automated workflows.

5 Prepare sample concentration of the cDNA.

6 Pipette 18 µl (9 µl) qPCR Mix into a precooled multiwell plate or precooled LightCycler® 8-Tube Strips.
– Add 2 µl (1 µl) of the cDNA template.
– Seal multiwell plate with a LightCycler® 480 Sealing Foil or LightCycler® 8-Tube Strips using the appropriate caps.

7 Place the Multiwell Plate 96 into a standard swinging-bucket centrifuge with a suitable adapter and balance it with a suitable counterweight (*e.g.*, another multiwell plate), or place the 8-Tube Strips into a standard Multiwell Plate 96 and balance them in the centrifuge.
– Centrifuge at 1,500 × *g* for 0.5 to 2 minutes.

8 Load the reaction vessels into the LightCycler® 480 Instrument.

9 Start the PCR program described above.
– If you use reaction volumes other than 20 µl, be sure to adapt the right volume in the running protocol. To start, we recommend using the same hold times as for the 20 µl volume.

3. Results

The following results were obtained using the EvoScript Universal cDNA Master with a synthetic transcript for β-Catenin gene according to the protocols described above. 140 to 1.4 × 10^8 (140 million) copies were reverse transcribed and 2 µl of cDNA was amplified with the LightCycler® Multiplex DNA Master in a LightCycler® 480 Instrument II.

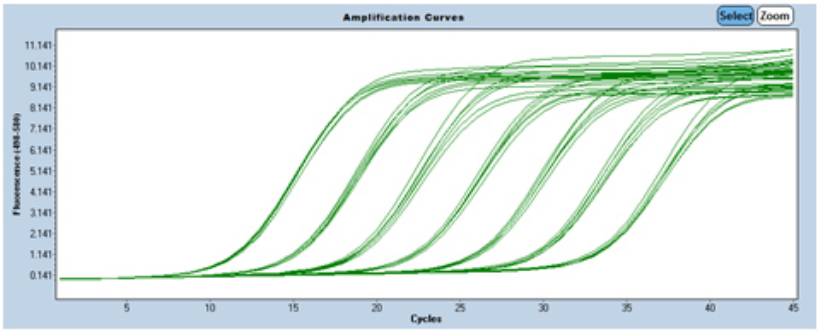

Fig. 1: Amplification curves of 140 (far right) to 1.4 × 10^8 (far left) copies of synthetic transcript for β-Catenin gene.

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. Use 10 ng to 5 µg of total RNA or 1 to 100 ng RNA.
		Add 10 mg/µl MS2 RNA to template to stabilize low concentrations of target RNA.
	Template RNA is degraded.	Prepare fresh RNA template, being careful to prevent RNase activity.
		Check RNA preparation by gel electrophoresis.
		Protect RNA from ribonuclease degradation by adding Protector RNase Inhibitor to the cDNA reaction. *Protector RNase Inhibitor concentrations up to 60 U will not interfere with RT-qPCR.*
	Too much template RNA.	A too high amount of template RNA may affect/ inhibit performance of RT reaction; decrease amount of RNA template.
	RT-PCR inhibitors are present in the RNA.	Make sure that the RNA is free of RT-PCR inhibitors, for example, use the Roche High Pure or MagNA Pure Kits for RNA purification and isolation.
	Reaction not optimized.	Both primers should have similar melting temperatures.
		Both primers should be present in the reaction at the same concentration.
		Try various primer concentrations (between 0.1 and 0.6 µM for each primer).
Nonspecific product bands.	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 EvoScript Universal cDNA Master provides a convenient solution for time-saving cDNA Synthesis for use in two-step, real-time RT-qPCR. All reagents needed for cDNA synthesis, including primers, nucleotides, buffers, and enzymes are supplied in only two vials, minimizing pipetting efforts. The enzyme blend included in this kit has a broad temperature range and is suitable for high temperature reverse transcription. The amount of random primer and oligo$(dT)_{18}$ primer included in the EvoScript Universal cDNA Master 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 Buffer* 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 EvoScript 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. The mix contains an optimized concentration of $Mg(OAc)_2$, eliminating the need for additional adjustments.

5.2. Quality Control

Each lot of the EvoScript Universal cDNA Master is tested to meet specifications of the two-step RT-qPCR using a thermal block cycler for the cDNA synthesis and the LightCycler® 480 Instrument II for the qPCR reaction 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.

6.3. Ordering Information

Roche 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 homepage lifescience.roche.com.

Product	Pack Size	Cat. No.
Accessories general (hardware)		
LightCycler® 480 Block Kit 96 Silver	1 block kit	05 015 219 001
LightCycler® 480 Block Kit 384 Silver	1 block kit	05 015 197 001
LightCycler® 8-Tube Strip Adapter Plate	1 piece, adapter plate, The adapter plate can be used multiple times	06 612 598 001
Accessories software		
LightCycler® 480 Software, Version 1.5	1 software package	04 994 884 001
Consumables		
LightCycler® 480 Multiwell Plate 96, white	5 x 10 plates	04 729 692 001
LightCycler® 480 Multiwell Plate 384, white	5 x 10 plates	04 729 749 001
LightCycler® 480 Sealing Foil	50 foils	04 729 757 001
LightCycler® 8-Tube Strips (white)	10x 12 white strips and clear caps.	06 612 601 001
Instruments		
LightCycler® 480 Instrument II	1 instrument	05 015 278 001
	1 instrument	05 015 243 001
MagNA Pure 96 Instrument		06 541 089 001
LightCycler® 96 Instrument	1 instrument	05 815 916 001
Reagents , kits		
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
MagNA Pure 96 Cellular RNA Large Volume Kit	1 kit, 3 sets, 3 x 96 isolations	05 467 535 001
MagNA Pure 96 DNA and Viral NA Small Volume Kit		06 543 588 001
MagNA Pure 96 DNA and Viral NA Large Volume Kit		06 374 891 001
RealTime ready Cell Lysis Buffer	1 bottle, 200 ml, 5,000 reactions of 40 µl final volume each	07 248 431 001
LightCycler® Multiplex DNA Master	1 kit, 1,000 reactions of 20 µl final volume each	07 339 577 001
	1 kit, 200 reactions of 20 µl final volume each	07 339 585 001

6.4. Trademarks

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All third party 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: http://technical-support.roche.com.

6.6. Regulatory Disclaimer

For general laboratory use.

6.7. Safety Data Sheet

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

6.8. Contact and Support

If you have questions or experience problems with this or any Roche product for Life Science, please contact our Technical Support staff. Our scientists are committed to providing rapid and effective help.
Please also contact us if you have suggestions for enhancing Roche product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to the research community worldwide.

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support** Site.

Visit **lifescience.roche.com**, to download or request copies of the following **Materials**:
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