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EvoScript RNA Master
5x concentrated

Version: 02
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Easy-to-use reaction mix for hot start one-step RT-qPCR.

Cat. No. 07 873 468 001 5 mL

Store the master at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	EvoScript RNA Master	Contains enzymes for reverse transcription and PCR, RT-qPCR Reaction Buffer, dATP, dCTP, dGTP, and dUTP, $Mg(OAc)_2$, and proprietary additives.	1 vial, 5 ml

1.2. Storage and Stability

Storage Conditions (Product)

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

Vial / bottle	Label	Storage
1	EvoScript RNA Master	Store at −15 to −25°C. ***Close lid immediately after use.*** ***Avoid temperature fluctuations (>5 times).*** *To avoid temperature fluctuations, aliquot and store at −15 to −25°C or store at +2 to +8°C for up to 4 weeks.*

Storage Conditions (Working Solution)

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

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Standard benchtop microcentrifuge
- Thermal block cycler or real-time PCR instrument
- Vortex mixer

For RT-qPCR

- Real-time PCR instrument, such as the LightCycler® 480 Instrument II*
- PCR reaction vessels, such as PCR tubes or microplates
- PCR primers
- Template RNA
- Water, PCR Grade*

1.4. Application

The EvoScript RNA Master is designed for sensitive, high-specificity, high-precision one-step RT-qPCR.
The master mix is ideally suited for easy reaction assembly requiring only the addition of primers, probe, and target RNA. It uses a hot start system that includes chemical modification and aptamer-mediated hot start. This enables highly specific priming for both, reverse transcription and DNA amplification[1]. The master mix is optimized for hydrolysis probes and does not require optimization with $Mg(OAc)_2$.

[1] The reverse transcription activity will be suppressed at temperatures significantly below +60°C.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Use any template RNA suitable for RT-qPCR in terms of purity, concentration, and absence of RT-PCR inhibitors.

Control Reactions

Negative control

Always run a negative control with the samples. To prepare a negative control, replace the template RNA with water. Contamination problems can be identified using negative control reactions.

Primers

Suitable concentrations of PCR primers range from 0.2 to 0.5 µM (final concentration in RT-qPCR). The recommended starting concentration is 0.2 µM each.

Probe

Suitable concentrations of hydrolysis probes range from 0.05 to 0.5 µM (final concentration in PCR). The recommended starting concentration is 0.1 µM each.

For best results, design hydrolysis probes with a higher Tm than the Tm of the assay primers.

General Considerations

$Mg(OAc)_2$

The master mix is optimized with a fixed concentration of $Mg(OAc)_2$, which works with nearly all primer combinations. There is no need for adjustment.

2.2. Protocols

Standard qPCR

Following the operator's manual of the instrument supplier, program the instrument with the following parameters:

Program Name	Cycles	Target	Hold [hh:mm:ss]	Acquisition Mode
Reverse transcription	1	60[1]	00:15:00	None
Initial denaturation	1	95	00:10:00	None
Amplification (2 steps)	45[2]	95	00:00:15	None
		58	00:00:30	Single
Cooling	1	40	00:00:30	None

[1] The EvoScript RNA Master includes a reverse transcriptase featuring hot start. The reverse transcription activity will be suppressed at temperatures significantly below +60°C.

[2] 45 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 RT-qPCR

Follow the procedure below to prepare one 20 µl standard reaction. Multiply the individual volumes with the appropriate factor for other reaction volumes.

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-concentrated solution of your primers and a 20x-concentrated solution of your probes.

3. In a 1.5 ml reaction tube, prepare the RT-qPCR Mix and place 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]	Final conc.
Water, PCR Grade*	9	–
EvoScript RNA Master, 5x conc.	4	1x
Primer Mix, 20x	1	1x
Probe Mix, 20x	1	1x
Total Volume	**15 µl**	

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 RNA template) is stable at +15 to +25°C for up to 4 hours, and is therefore ideal for use in automated workflows.

5. Prepare the RNA sample.

6. Pipette 15 µl RT-qPCR Mix into a precooled PCR tube or a precooled well of a multiwell plate, depending on your real-time PCR instrument.

7. Add 5 µl of the RNA template.

8. Prepare and centrifuge the PCR tubes or multiwell plate according to the instructions supplied with your instrument.

3. Additional Information on this Product

3.1. Quality Control

Each lot of EvoScript RNA Master is tested to meet specifications of the RT-qPCR using a RT-qPCR assay on the LightCycler® 480 Instrument II*.

4. Supplementary Information

4.1. 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.

4.2. Changes to previous version

Layout changes.
Editorial changes.

4.3. Trademarks

EVOSCRIPT and LIGHTCYCLER are trademarks of Roche.
All other product names and trademarks are the property of their respective owners.

4.4. License Disclaimer

Consult product detail pages at **custombiotech.roche.com** for patent license limitations, if available.

4.5. Regulatory Disclaimer

For further processing only.

4.6. Safety Data Sheet

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

4.7. Contact and Support

For additional documentation such as certificates and safety data sheets, please visit **documentation.roche.com**.

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