EvoScript Reverse Transcriptase

Easy-to-use reaction mix for reverse transcription

Cat. No. 07 912 315 001 1 kit up to 50 reactions of 20 µl final volume each
Cat. No. 07 912 323 001 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

<table>
<thead>
<tr>
<th>Vial / Bottle</th>
<th>Cap</th>
<th>Label</th>
<th>Function / Description</th>
<th>Catalog Number</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>blue</td>
<td>EvoScript Reverse Transcriptase, Reverse Transcriptase</td>
<td>Contains Protector RNase Inhibitor and Enzyme Blend, 10x conc.</td>
<td>07 912 315 001</td>
<td>1 vial, 100 μl</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>07 912 323 001</td>
<td>4 vials, 100 μl each</td>
</tr>
<tr>
<td>2</td>
<td>red</td>
<td>EvoScript Reverse Transcriptase, Reaction Buffer</td>
<td>Contains dNTPs, Mg(OAc)₂, and salts, 5x conc.</td>
<td>07 912 315 001</td>
<td>1 vial, 200 μl</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>07 912 323 001</td>
<td>4 vials, 200 μl each</td>
</tr>
<tr>
<td>3</td>
<td>colorless</td>
<td>EvoScript Reverse Transcriptase, Water, PCR Grade</td>
<td>To adjust the final reaction volume.</td>
<td>07 912 315 001</td>
<td>1 vial, 1 ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>07 912 323 001</td>
<td>4 vials, 1 ml each</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Vial / Bottle</th>
<th>Cap</th>
<th>Label</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>blue</td>
<td>Reverse Transcriptase</td>
<td>Store the kit at −15 to −25°C or store at +2 to +8°C for a maximum of 4 weeks.</td>
</tr>
<tr>
<td>2</td>
<td>red</td>
<td>Reaction Buffer</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>colorless</td>
<td>Water, PCR Grade</td>
<td></td>
</tr>
</tbody>
</table>

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
- Gene-specific primer for the reverse transcription reaction

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. General Information

1.4. Application

The EvoScript Reverse Transcriptase is designed for highly sensitive and convenient cDNA synthesis in two-step real-time 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 gene-specific primers and target RNA.

1.5. Preparation Time

Assay Time

<table>
<thead>
<tr>
<th>Step</th>
<th>Time [min]</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT reaction setup time</td>
<td>30</td>
</tr>
<tr>
<td>RT reaction</td>
<td>30</td>
</tr>
<tr>
<td>PCR reaction setup time</td>
<td>30</td>
</tr>
<tr>
<td>PCR reaction</td>
<td>120</td>
</tr>
<tr>
<td>Total Time</td>
<td>3 hours 30 minutes</td>
</tr>
</tbody>
</table>
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.

ℹ️ RNases can also be present on contaminated glassware.


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.

Primers

Suitable concentrations of gene-specific RT primer range from 0.5 to 2.5 μM (recommended final concentration is 2 μM).

General Considerations

Precautions

Use RNase-free techniques. RNase-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 RNase carryover.
- Use only reagents provided in this kit. Substitutions may introduce RNases.
- Clean and decontaminate work areas and instruments, including pipettes, with commercially available decontamination reagents.
- Use only new RNase-free aerosol-blocking pipette tips and microcentrifuge tubes.
- Use a work area specifically designated for RNA work, and if possible, use reaction vessels and pipettes dedicated only for work with template RNA.
2. How to Use this Product

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

Setup of the cDNA Synthesis

1. Gene-specific RT primer is not provided with this kit.

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:

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Water, PCR Grade (Vial 3)</td>
<td>X</td>
<td>–</td>
</tr>
<tr>
<td>Reaction Buffer, 5x conc.</td>
<td>4.0</td>
<td>1x</td>
</tr>
<tr>
<td>Gene-Specific RT Primer</td>
<td>X</td>
<td>0.5 to 2.5 μM</td>
</tr>
<tr>
<td>Template RNA</td>
<td></td>
<td>2.5 μg (down to 1 pg)</td>
</tr>
</tbody>
</table>

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 primer 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.

(*) 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.
2. How to Use this Product

Standard Reverse Transcription Protocol

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

1. Heat to +65°C for 30 minutes.

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

3. 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 without purification to a PCR with sequence-specific primers. 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.

For reaction details and recommendations, please refer to the Instructions for Use of the PCR Master you use.
### 3. Troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible cause</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No PCR product or very little amount of PCR</td>
<td>Insufficient amount of template RNA.</td>
<td>Check quality and concentration of template.</td>
</tr>
<tr>
<td>product.</td>
<td></td>
<td>Increase amount of RNA template in cDNA reaction (maximum 2.5 μg/20 μl reaction).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Add 10 μg/μl MS2 RNA to template to stabilize low concentrations of target RNA.</td>
</tr>
<tr>
<td></td>
<td>Template RNA is degraded.</td>
<td>Prepare fresh RNA template, being careful to prevent contamination with RNases.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Check RNA preparation by gel electrophoresis.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Add Protector RNase Inhibitor® to the cDNA synthesis step.</td>
</tr>
<tr>
<td></td>
<td>Too much template RNA.</td>
<td>A too high amount of template RNA may affect/inhibit performance of RT-PCR; decrease amount of RNA template.</td>
</tr>
<tr>
<td></td>
<td>RT-PCR inhibitors are present in the RNA.</td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td>Reaction not optimized.</td>
<td>Both primers should have similar melting temperatures.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Both primers should be present in the reaction at the same concentration.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Try various primer concentrations (between 0.1 and 0.6 μM for each primer).</td>
</tr>
<tr>
<td>Nonspecific PCR Products.</td>
<td>Contaminating DNA in sample.</td>
<td>Perform a control without reverse transcription step; for details, see section on DNA contamination.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td>Inhibitors of RT reaction.</td>
<td>Remove inhibitors by precipitating the mRNA, washing the precipitate with 70% ethanol, and then redissolving the precipitate.</td>
</tr>
<tr>
<td></td>
<td>Too much cDNA template in PCR reaction.</td>
<td>Dilute cDNA before use in real-time PCR.</td>
</tr>
</tbody>
</table>
4. Additional Information on this Product

4.1. Test Principle

The EvoScript Reverse Transcriptase provides a convenient solution for time-saving cDNA synthesis for use in two-step, real-time RT-PCR. All reagents needed for cDNA synthesis, except gene-specific RT primer, but including nucleotides, buffers, and enzyme blend 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 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 Reverse Transcriptase enables reliable cDNA synthesis over a wide dynamic range even for GC-rich templates, and is ideal for high-throughput quantitative RT-PCR analysis.

4.2. Quality Control

Each lot of the EvoScript Reverse Transcriptase 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.
5. Supplementary Information

5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

<table>
<thead>
<tr>
<th>Text convention and symbols</th>
<th>Information Note: Additional information about the current topic or procedure.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Important Note: Information critical to the success of the current procedure or use of the product.</td>
</tr>
<tr>
<td>1 2 3 etc.</td>
<td>Stages in a process that usually occur in the order listed.</td>
</tr>
<tr>
<td>1 2 3 etc.</td>
<td>Steps in a procedure that must be performed in the order listed.</td>
</tr>
<tr>
<td>* (Asterisk)</td>
<td>The Asterisk denotes a product available from Roche Diagnostics.</td>
</tr>
</tbody>
</table>

5.2. Changes to previous version

Layout changes.
Editorial changes.
Number of vials in the content table have been corrected.

5.3. 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.

<table>
<thead>
<tr>
<th>Product</th>
<th>Pack Size</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instruments</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MagNA Pure 96 Instrument</td>
<td></td>
<td>06 541 089 001</td>
</tr>
<tr>
<td>Reagents, kits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mRNA Isolation Kit for Blood/Bone Marrow</td>
<td>1 kit, 30 [100] isolations from 5 ml [1.5 ml] sample volumes</td>
<td>11 934 333 001</td>
</tr>
<tr>
<td>RNA, MS2</td>
<td>500 μl, 10 A260 units</td>
<td>10 165 948 001</td>
</tr>
<tr>
<td>Protector RNase Inhibitor</td>
<td>2,000 U, (40 U/μl)</td>
<td>03 335 399 001</td>
</tr>
<tr>
<td></td>
<td>10,000 U, 5 x 2,000 U</td>
<td>03 335 402 001</td>
</tr>
<tr>
<td>mRNA Isolation Kit</td>
<td>1 kit</td>
<td>11 741 985 001</td>
</tr>
<tr>
<td>High Pure RNA Isolation Kit</td>
<td>1 kit, 50 isolations</td>
<td>11 828 665 001</td>
</tr>
<tr>
<td>High Pure RNA Tissue Kit</td>
<td>1 kit, 50 isolations</td>
<td>12 033 674 001</td>
</tr>
<tr>
<td>High Pure RNA Paraffin Kit</td>
<td>1 kit, up to 100 isolations</td>
<td>03 270 289 001</td>
</tr>
<tr>
<td>MagNA Pure 96 Cellular RNA Large Volume Kit</td>
<td>1 kit, 3 sets, 3 x 96 isolations</td>
<td>05 467 535 001</td>
</tr>
<tr>
<td>MagNA Pure 96 DNA and Viral NA Small Volume Kit</td>
<td></td>
<td>06 543 588 001</td>
</tr>
<tr>
<td>MagNA Pure 96 DNA and Viral NA Large Volume Kit</td>
<td></td>
<td>06 374 891 001</td>
</tr>
<tr>
<td>RealTime ready Cell Lysis Buffer</td>
<td>1 bottle, 200 ml, 5,000 reactions of 40 μl final volume each</td>
<td>07 248 431 001</td>
</tr>
<tr>
<td>LightCycler® Multiplex DNA Master</td>
<td>1 kit, 1,000 reactions of 20 μl final volume each</td>
<td>07 339 577 001</td>
</tr>
<tr>
<td></td>
<td>1 kit, 200 reactions of 20 μl final volume each</td>
<td>07 339 585 001</td>
</tr>
</tbody>
</table>
5.4. Trademarks

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

5.5. License Disclaimer

For patent license limitations for individual products please refer to: http://technical-support.roche.com.

5.6. Regulatory Disclaimer

For general laboratory use.

5.7. Safety Data Sheet

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

5.8. 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:
- Instructions for Use
- Safety Data Sheets
- Certificates of Analysis
- Information Material

To call, write, fax, or email us, visit lifescience.roche.com and select your home country to display country-specific contact information.