For further processing only.

# NxtScript 2G RT, conc.

## thermostable reverse transcriptase, 500 U/µL, glycerol-free solution

**Version: 02**

Content Version: February 2023

Lyo-ready reverse transcriptase for RT-qPCR at high temperatures.

| | |
|---|---|
| **Cat. No. 09 085 220 103** | custom fill<br>500 U/µL |

**Store the product at −15 to −25°C.**

# 1. General Information

## 1.1. Contents

| Vial / Bottle | Label | Function / Description | Content |
|---|---|---|---|
| 1 | NxtScript 2G RT, conc. | Enzyme supplied in glycerol-free solution. | Custom fill |

## 1.2. Storage and Stability

### Storage Conditions (Product)

When stored at −15 to −25°C, the product is stable through the expiry date printed on the label.

| Vial / Bottle | Label | Storage |
|---|---|---|
| 1 | NxtScript 2G RT, conc. | Store at −15 to −25°C.<br>Aliquot the enzyme into polypropylene tubes after first use and store at −15 to −25°C. Low protein binding tubes may affect product stability.<br>⚠ ***Close lid immediately after use.***<br>⚠ ***Avoid repeated freezing and thawing (more than 5 times).*** |

## 1.3. Additional Equipment and Reagent required

**Standard laboratory equipment**

- Nuclease-free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Sterile, RNase-free reaction tubes for preparing PCR mixes and dilutions
- Sterile, RNase-free PCR reaction vessels, such as thin-walled PCR tubes or plates
- Standard benchtop microcentrifuge
- Thermal block cycler with heated lid

**For cDNA synthesis**

- Gene-specific primer or oligo(dT) for reverse transcription reaction
- Reaction buffer containing 50 mM tricine, 50 mM potassium acetate, 6 mM magnesium acetate, pH 8.3
- dNTPs
- PCR-grade water
- RNase inhibitor (optional)

# 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 10 pg up to 1 µg total RNA.

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

For reproducible isolation of nucleic acids, use:

- RNase inhibitors or other isolation conditions that inactivate RNases.
- If necessary, analyze different steps in the process, such as lysis or isolation by gel electrophoresis (ethidium bromide staining) to ensure that the sample remains RNase-free.
- Avoid any contamination with RNases from other potential sources, such as glassware, plasticware, reagent solutions, and hands.

### Control Reactions

Always run a negative control with the samples.

- To prepare a negative control, replace the template DNA with PCR-grade water to reveal contamination problems (no template control).
- 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

#### Primers for RT

A suitable concentration of gene-specific RT primers is 500 nM.

⚠ ***To assure optimum performance of NxtScript 2G RT, conc., always use highly purified PCR primers, for example, by HPLC. Optimize the primer concentration first, then determine the probe optimization using the optimized primer concentrations.***

The optimal primer concentration is the lowest concentration that results in the lowest Cq and an adequate fluorescence for a given target concentration.

### General Considerations

#### Precautions

Use RNase-free techniques. RNase-contaminated reagents and reaction vessels may degrade template RNA. Follow these guidelines to the minimize risk of contamination:

- Wear disposable gloves and change them frequently.
- Avoid touching surfaces or materials that could cause RNase carryover.
- 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.

# 2.2. Protocols

## cDNA synthesis

### Purified RNA as template

Use up to 1 µg RNA/20 µL reaction if purified RNA is used as template for the cDNA synthesis.

⚠ ***If >1 µg RNA was used per 20 µL cDNA synthesis reaction, do not use more than 10% 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 and hydrolysis probes assays.***

### Setup of the cDNA synthesis

⚠ ***Program the instrument before preparing the reaction mixes.***

*i The following protocol provides sufficient reaction mix for 10 reactions.*

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

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

   *i See section,* **Additional Equipment and Reagent Required** *for additional information on preparing Reaction buffer.*

| Reagent | Volume for 10 reactions [µL] | Final conc. |
|---|---|---|
| Water, PCR grade | X | – |
| Reaction buffer | X | 1x |
| Gene-specific RT primer or oligo(dT) | X | 0.5 – 2.5 µM |
| dNTPs | X | 0.5 – 1.5 mM each |
| Template RNA | X | 1 µg (down to 1 pg)[1] |
| NxtScript 2G RT, conc.[2] | 2 | 1x |
| **Total Volume** | **200** | |

3. Mix 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.

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

[2] The volume mentioned in the table is a general recommendation, with 1 µL per 10 reactions being the minimum recommended volume. The exact amount of the enzyme needed may vary depending on the experimental conditions and should be individually determined.

### Standard reverse transcription protocol

The reverse transcription protocol shown below is optimized for the NxtScript 2G RT, conc.

1. Heat to +65°C for 10 to 30 minutes.

   *i The optional temperature range is +55 to +70°C.*

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

3. Stop the reaction by placing the tube on ice.
   – 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.

*i Optional: In case of longer standing times at temperatures >4°C post the reverse transcription reaction, an additional denaturation step of 95°C for 5 min may be performed to inactivate the enzyme.*

## PCR reaction protocol

The resulting cDNA can be added without purification to a PCR with sequence-specific primers.

1. For initial experiments on one of the LightCycler® Instruments, use 2 µL of the cDNA reaction or dilutions of it, in a 20 µL reaction.

*For additional information, see the Instructions for Use of the PCR Master you use.*

# 3. Results

The following results were obtained using NxtScript 2G RT, conc. in a singleplex reaction using primers and hydrolysis probe (FAM) specific Human Reference total RNA (β2M) on the LightCycler® 480 Instrument II.

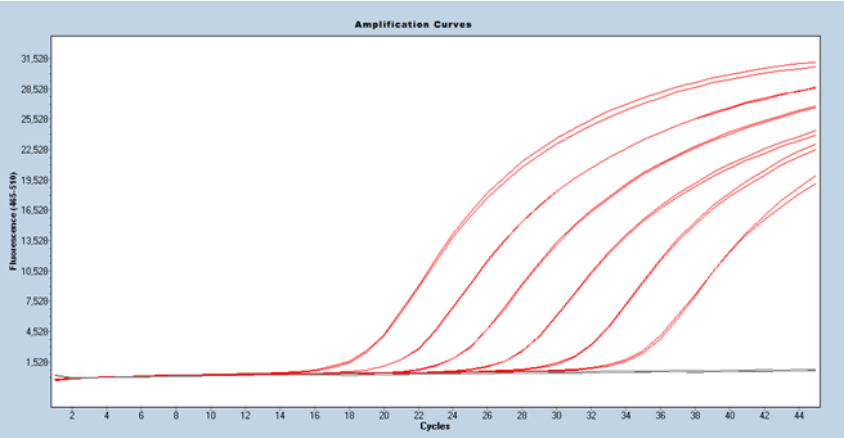


**Fig. 1:** The FAM channel shows the results for β2M. Amplification curves shown in red were obtained from dilutions with 1,000 ng (far left), 100 ng, 10 ng, 1 ng, 0.1 ng, and 0.01 ng (far right) human total RNA per 20 µL/well RT reaction including a no template control (flat line). qPCR was performed in a 20 µL reaction with 2 µL cDNA from a previous RT reaction in a LightCycler® 480 Multiwell Plate 96.

# 4. Troubleshooting

| Observation | Possible cause | Recommendation |
|---|---|---|
| Little or no PCR product. | Insufficient amount of template RNA. | Check quality and concentration of template. |
| | | Increase amount of RNA template in cDNA reaction (maximum 1 µg/20 µL reaction). |
| | Template RNA is degraded. | Prepare fresh RNA template, being careful to prevent contamination with RNases. |
| | | Check RNA preparation by gel electrophoresis. |
| | | Add 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. |
| | RT-PCR inhibitors are present in the RNA. | Make sure that the RNA is free of RT-PCR inhibitors. |
| | 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 0.5 and 2.5 µM for each primer). |
| Nonspecific PCR products present. | Contaminating DNA in sample. | Perform a control without reverse transcription step; for details, see section, **Control Reactions**. |
| | | 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. |
| | Too much cDNA template in PCR reaction. | Dilute cDNA before use in real-time PCR. |
| Negative control sample gives a positive signal. | Contamination present. | Remake all critical reaction mixes. |
| | | Use special PCR setup working areas. |
| | | Use a new tube of Water, PCR Grade. |

# 5. Additional Information on this Product

## 5.1. Quality Control

For lot-specific certificates of analysis, see section, **Contact and Support**.

# 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

Editorial changes that provide further clarification on existing protocol:

Additional foot note (2) added to section **2.2 Protocols, subsection Setup of cDNA synthesis, step 2 , NxtScript 2G RT, conc.** Foot notes (2) states: The volume mentioned in the table is a general recommendation, with 1 µL per 10 reactions being the minimum recommended volume. The exact amount of the enzyme needed may vary depending on the experimental conditions and should be individually determined.

Additional information on **(i) optional step provided under section 2.2 Protocols subsection Standard reverse Transcription protocol, after step 3.** Additional information states : (i) Optional: In case of longer standing times at temperatures >4°C post the reverse transcription reaction, an additional denaturation step of 95°C for 5 min may be performed to inactivate the enzyme.

## 6.3. Trademarks

NXTSCRIPT 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.

## 6.4. License Disclaimer

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

## 6.5. Safety Data Sheet

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

## 6.6. Contact and Support

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

**Your Roche CustomBiotech Customer Service:**

**Europe, Middle East, Africa and Latin America**
Roche Diagnostics Deutschland GmbH
Phone +49 621 759 8580
Fax +49 621 759 6385
mannheim.custombiotech@roche.com

**United States**
Roche Diagnostics Corporation
Phone +1 800 428 5433 (toll free)
Fax +1 317 521 4065
custombiotech.ussales@roche.com

**Canada**
Roche Diagnostics
Phone +1 450 686 7050
Fax +1 450 686 7012
custombiotech.can@roche.com

**Japan**
Roche Diagnostics K.K.
Phone +81 3 6634 1046
Fax +81 3 5479 0585
japan.custombiotech@roche.com

**Asia Pacific**
Roche Diagnostics Asia Pacific Pte. Ltd.
Phone +65 6371 6638
Fax +65 6371 6601
apac.custombiotech@roche.com

0223.09094440001 ②

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany