For further processing into IVD products and medical devices only.

NxtScript DNA Master
5x concentrated

Version: 05
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Easy-to-use reaction mix optimized for multiplex qPCR and qRT-PCR.

Cat. No. 07 368 143 103	5 mL 5x conc.

Store the master at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	NxtScript DNA Master, 5x conc.	▪ Ready-to-use hot start qPCR Mix. ▪ Contains qPCR Reaction Buffer, AptaTaq Polymerase, dATP, dCTP, dGTP, and dUTP, $MgCl_2$, and proprietary additives.	1 vial, 5 ml

1.2. Storage and Stability

Storage Conditions (Product)

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

Vial / bottle	Label	Storage
1	NxtScript DNA Master, 5x conc.	Store the master at +2 to +8°C for a maximum of 4 weeks, or store in aliquots at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing (more than 5 times).***

Storage Conditions (Working Solution)

Although we recommend working on ice and preparing the reagents immediately before use, a prepared mix containing NxtScript DNA Master, primers, and probe is stable at +15 to +25°C for up to 4 hours.

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Autoclaved PCR reaction vessels, such as PCR tubes or microplates
- Standard benchtop microcentrifuge
- Thermal block cycler
- Vortex mixer

For standard qPCR

- PCR primers
- Template DNA
- Water, PCR Grade*

For standard qRT-PCR

- NxtScript Reverse Transcriptase*
- NxtScript 2G RT, conc.*
- PCR primers
- Template RNA
- Water, PCR Grade*

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Use any DNA suitable for qPCR or template RNA, such as total RNA or mRNA suitable for RT-PCR in terms of purity, concentration, and absence of inhibitors. RNA and DNA purified from whole blood, plasma, serum, swabs, stool, and urine were tested successfully with NxtScript DNA Master.

Control Reactions

Negative control

Always run a negative control with the samples. To prepare a negative control, replace the template DNA or RNA with Water, PCR Grade*.

Primers

Primer concentration

Use PCR primers at a final concentration of 0.2 to 0.5 µM. The recommended concentration is 0.5 µM each.
⚠ ***Always use equimolar primer concentrations.***

Probe

As a starting point, use a probe concentration of 0.25 µM each. However, suitable concentrations range from 0.05 to 0.5 µM.
i *The optimal probe concentration is the lowest concentration that results in the lowest quantification cycle value (Cq) and adequate fluorescence dynamics for a given target concentration.*
i *To ensure efficient probe cleavage, the Tm of the hydrolysis probe should be higher than the Tm of the primers.*

General Considerations

The optimal reaction conditions, such as concentration of template DNA or RNA, concentration of PCR primers, incubation temperatures and times, and cycle number depend on the specific template/primer system and must be determined individually.

2.2. Protocols

Standard qPCR protocol

PCR setup

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 an autoclaved reaction tube, prepare the qPCR mix and place on ice.
– For best results, prepare at least 10 reactions in order to reduce pipetting errors.

Reagent	Volume [µl] 1 Reaction	Final conc.
NxtScript DNA Master, 5x conc.	4	1x
Primer mix, 20x conc.	1	1x
Probe mix, 20x conc.	1	1x
Water, PCR Grade*	9	–
Total Volume	**15 µl**	

To prepare the mix for more than one reaction, multiply the amount in the "Volume 1 Reaction" column by the number of reactions to be run, plus at least one additional reaction.

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

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

5 Prepare sample concentration of the DNA.

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

7 Add 5 µl of the DNA template.

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

9 Centrifuge the PCR tubes or multiwell plate if required.

Standard qPCR

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

Cycles	Analysis Mode	Target Temperature [+°C]	Hold Time [sec]	Program Name
1	None	95	30	Initial denaturation
45[1]	Quantification	95	5	Amplification
		60[2]	30	
1	None	4	–	Cooling

2 Place your tubes or plate in the instrument and start the reaction.

3 When the real-time PCR is finished, follow the instrument instructions for quantification and data analysis.

[1] 45 cycles are 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.

[2] The NxtScript DNA Master includes an aptamer for hot start. Anneal/Extend temperatures significantly below +60°C may not be well tolerated because aptamers will inhibit DNA polymerase activity at low temperatures.

Standard qRT-PCR protocol

qRT-PCR setup

For qRT-PCR, use NxtScript Reverse Transcriptase*. NxtScript Reverse Transcriptase is a designed reverse transcriptase with higher stability than native reverse transcriptase. This feature allows for higher reverse transcription temperatures up to +60°C.
Follow the procedure below to prepare at least ten 20 µl standard reactions. 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 an autoclaved reaction tube, prepare the RT-qPCR mix and place on ice.

Reagent	Volume [µl] 1 Reaction	Final conc.
NxtScript DNA Master, 5x conc.	4	1x
NxtScript Reverse Transcriptase*[1]	X (5 – 20 U)	–
Primer mix, 20x conc.[2]	X	0.2 – 0.5 µM
Probe mix, 20x conc.	X	0.05 – 0.5 µM
Water, PCR Grade*	X	–
Total Volume	**15 µl**	

To prepare the mix for more than one reaction, multiply the amount in the "Volume 1 Reaction" column by the number of reactions to be run, plus at least one additional reaction.

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

5. Prepare sample concentration of the RNA.

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

7. Add 5 µl of the RNA template.

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

9. Centrifuge the PCR tubes or multiwell plate if required.

[1] Note that higher concentrations of the reverse transcriptase might result in lower Cq due to inhibition of the subsequent PCR reaction.
[2] Due to possible primer/primer interactions that occur during storage, it may be necessary to preheat the PCR primer mix for 1 minute at +95°C before pipetting the mixture. This extra step will ensure optimum sensitivity.

Standard qRT-PCR

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

Cycles	Analysis Mode	Target Temperature [+°C]	Hold Time	Program Name
1	None	45 – 55[1]	4 – 10 min[2]	Reverse transcription
1	None	95	30 sec	Initial denaturation
45	Quantification	95	5 sec	Amplification
		60[3]	30 sec	
1	None	4	–	Cooling

2. Place your tubes or plate in the instrument and start the reaction.

3. When the real-time PCR is finished, follow the instrument instructions for quantification and data analysis.

[1] We suggest running the reverse transcription at +45 to +55°C. If necessary, it is also possible to run it at +60°C.
[2] Ten minutes is the suggested default protocol. Depending on your assay, it should be possible to reduce the time for reverse transcription down to 4 minutes. For best results, run a test with 10, 8, 6, 4 minutes, etc.
[3] The NxtScript DNA Master includes an aptamer for hot start. Anneal/Extend temperatures significantly below +60°C may not be well tolerated because aptamers will inhibit DNA polymerase activity at low temperatures.

3. Additional Information on this Product

3.1. Quality Control

Each lot of NxtScript DNA Master is tested to meet specifications of the qPCR using a duplex 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.
1. 2. 3. 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

Updated regulatory disclaimer.

4.3. Trademarks

NXTSCRIPT, APTATAQ 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 into IVD products and medical devices 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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