One-Step RT-qPCR 2X GreenMastermix

With SybrGreen®

Component	Cat#	SM3137.0200	SM3137.0500	SM3137.1000	Colour code of cap
One-Step RT-qPCR 2X GreenMastermix		2x 1mL	5x 1.mL	10x 1mL	

Description

The **Genaxxon One-Step RT-qPCR 2X GreenMastermix** was developed for quantitative real-time analyzes of RNA templates using **SybrGreen®**. The ready-to-use mixture is based on a genetically modified **reverse transcriptase** with improved thermal stability, which offers increased specificity, a high cDNA yield and improved efficiency for highly structured and long cDNA fragments.

The 2X Mastermix contains all the reagents required for the RT-qPCR (except for the template and primer) to ensure quick and easy handling at a minimum of pipetting steps. The **high-quality enzymes** and the **optimized reaction buffer with high-purity dNTPs** guarantee superior real-time PCR results.

This Master Mix does not contain ROX - please make sure to choose the right setting on your qPCR cycler.
For qPCR cyclers requiring ROX as a reference dye, One-Step RT-qPCR 2X Greenmastermix is also available with ROX - just ask us: info@genaxxon.com.

Product specifications

Concentration:	2 time ready-to-use master mix
Reverse Transcriptase	Yes
Hot Start Polymerase	Yes
RNase Inhibitor	Yes
ROX®	No

Advantages

Ultra-Fast – RT-qPCR in <35 minutes
One-step RT-qPCR – One tube format
With SybrGreen
No need for Superscript!
Money saving – high!

Shipment: on wet ice but full activity at RT (+15°C to +25°C) for at least 1 week

Short-Term Storage: Can be stored at +2°C to +8°C for the duration of use within weeks to reduce freeze-thaw stress
Long-Term Storage: Stored at -20°C protected from light

Shelf-life: Stable until expiration date when stored and handled correctly

Important notes before getting started

- This protocol is a guideline; for maximum efficiency and specificity, adjustments of annealing temperature as well as extension time, primer concentration and template concentration may be needed.
- For most RT-qPCR assays, standard cycling conditions can be used without modification. However, cycling conditions are highly dependent on factors such as primers, amplicons, and input material, so adjustments may be necessary in some cases.
- Ultra-fast cycling conditions are largely influenced by the ramping rate of your qPCR cycler, as well as the primers, amplicons, and input material, so adjustments may be necessary. In most cases, ultra-fast cycling conditions can be used without modification, as long as the primer sets do not exhibit non-specific binding.

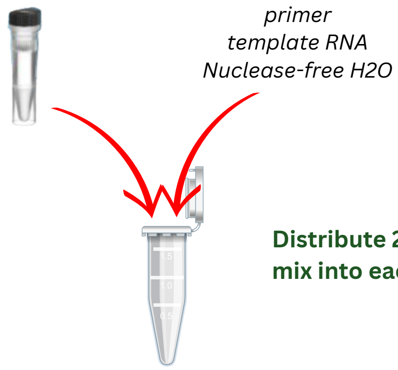
PCR Protocol

6-8 times

Thaw One-Step RT-qPCR 2X GreenMastermix.

Invert tube 6-8 times.

Do not vortex!
This may harm the enzymes!



Distribute 20µL reaction mix into each tube.

PCR protocol

Components	Quantities
One-Step RT-qPCR 2X GreenMastermix	10 µL
primer 1 (8 µM stock concentration):	1 µL (400 nM; 100 - 600 nM recommended)
primer 2 (8 µM stock concentration):	1 µL (400nM; 100 - 600 nM recommended)
Template RNA	variable volume (0.1 - 100ng/reaction)
Nuclease-free water	up to 20 µL

Choose Standard PCR-Protocol or Ultra-FAST PCR-Protocol

Standard PCR-Protocol

With additional annealing or...

Place PCR tubes in the thermal cycler* and start program.

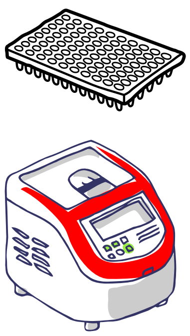
A typical PCR cycling program is outlined on the right For maximum yield and specificity, temperatures and cycling times should be optimized.

One-Step RT-qPCR Protocol
with additional annealing

Step	Time	Temperature
Reverse Transcription	10 min	50 °C
Initial denaturation	3 min	92 - 95 °C
Denaturation	5 sec	92 - 95 °C
Annealing	5 sec	60°C*
Extension	5 - 10 sec	72 °C
Number of Cycles	25 - 45	

*Depending on primer

...without additional annealing.



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A typical PCR cycling program is outlined on the right For maximum yield and specificity, temperatures and cycling times should be optimized.

One-Step RT-qPCR Protocol
without additional annealing

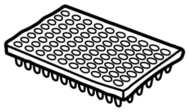
Step	Time	Temperature
Reverse Transcription	10 min	50 °C
Initial denaturation	3 min	92 - 95 °C
Denaturation	5 sec	92 - 95 °C
Annealing/Extension	10 sec	60°C*
Number of Cycles	25 - 45	

*Depending on primer

We Aim for Your Success.

Ultra-Fast Protocol

With additional annealing or...



Place PCR tubes in the thermal cycler* and start program.

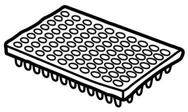
A typical PCR cycling program is outlined on the right For maximum yield and specificity, temperatures and cycling times should be optimized.

Ultra-Fast RT-qPCR Protocol
with additional annealing

Step	Time	Temperature
Reverse Transcription	5 min	50 °C
Initial denaturation	1 min	92 - 95 °C
Denaturation	1 sec	92 - 95 °C
Annealing	1 sec	60°C*
Extension	1 - 5 sec	72 °C
Number of Cycles	25 - 45	

*Depending on primer

...without additional annealing.



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Step	Time	Temperature
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Additional information

Applications

- **Dye-based quantitative PCR**
- **RNA and miRNA Expression**
- **High-Resolution Melting Curve Analysis**
- **Transcript Variant Analysis**

Quality Control

The One-Step RT-qPCR 2X GreenMastermix undergoes rigorous quality control. Each lot is tested in a probe-based qPCR using both RNA and MS2 Phage RNA as input and must meet the standards defined in our quality control chart. Enzyme purity and homogeneity of over 98% are verified by Bioanalyzer SDS protein electrophoresis.
The One-Step RT-qPCR 2X GreenMastermix is free of detectable endonuclease- & exonuclease activity

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online as pdf-file or on request (info@genaxxon.com).

Product Use Limitations

One-Step RT-qPCR 2X GreenMastermix is developed, designed, and sold for research purposes only. It is not to be used for human, diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this manual.

Further information

For more information, please visit our website: www.genaxxon.com
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