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One-Step RT-qPCR (2X) master mix - Probe

Product	Cat#	Package size
One-Step RT-qPCR 2-time master mix - Probe Master mix incl. all ingredient except for primer and RNA template.	**M3138.0250**	**250 reactions x 20µL (2 x 1.25mL)**
One-Step RT-qPCR 2-time master mix - Probe Master mix incl. all ingredient except for primer and RNA template.	**M3138.1250**	**1250 reactions x 20µL (10 x 1.25mL)**

Product description

The Genaxxon bioscience One-Step RT-qPCR 2X master mix – Probe is designed for quantitative real-time analyses of RNA templates using Dual Labeled Fluorescent Probes. The ready-to-use mix is based on a genetically engineered reverse transcriptase with enhanced thermal stability providing increased specificity, high cDNA yield and improved efficiency for highly structured and long cDNA fragments.

The 2X master mix contains all reagents required for RT-qPCR (except template, primers and the dual labelled fluorescent probe) to ensure fast and easy preparation with a minimum of pipetting steps. The highest quality enzymes and the optimized reaction buffer containing our ultrapure dNTPs ensure superior real time PCR results.

RT-qPCR is used to amplify double-stranded DNA from single stranded RNA templates to allow a rapid real-time quantification of RNA targets. In the reverse transcription step the reverse transcriptase synthesizes single-stranded DNA molecules (cDNA) complementary to the RNA template. In the first cycle of the PCR step the Hotstart DNA polymerase synthesizes DNA molecules complementary to the cDNA, thus generating a double-stranded DNA template. The Hotstart polymerase activity is blocked at ambient temperature and switched on automatically at the onset of the initial denaturation. The thermal activation prevents the extension of non-specifically annealed primers and primer-dimer formations at low temperatures during PCR setup.

During subsequent rounds of cycling the DNA polymerase exponentially amplifies this double-stranded DNA template. In one-step RT-PCR all components of RT and PCR are mixed in one tube so that the complete reaction can be performed without opening the tube once the reaction was started. This offers tremendous convenience when applied in routine testing and minimizes the risk of contaminations.

The One-Step RT-qPCR master mix contains RNase inhibitor that is essential when working with low amounts of starting RNA.

The premium quality Reverse Transcriptase, ultrapure dNTPs and an optimized reaction buffer ensure superior results with highest reproducibility. The kit is optimized for high efficiency in a broad range of primer-template combinations.

Dual labelled fluorescent probes:

Real-time PCR technology based on dual labelled DNA probes provides a highly sensitive and specific PCR system with multiplexing capability. It requires two standard PCR primers and the DNA probe that hybridizes to an internal part of the amplicon. The sequence of the dual labelled DNA probe should avoid secondary structure and primer-dimer formation.

Sensitivity:

Targets can generally be detected from <1pg to 20ng poly(A) RNA (mRNA) or 10pg to 1µg total RNA. Even lower amounts of RNA may be successfully amplified by using highly expressed transcripts.

Supplied buffers/solutions

- One-Step RT-qPCR 2X master mix – Probe containing reverse transcriptase, antibody-blocked Hotstart polymerase, RNase inhibitor, dNTPs, reaction buffer, additives and stabilizers.

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Quality Control

Nuclease activity: 50ng of radio labelled DNA or RNA is incubated with 200 units of the enzyme in 1X reaction buffer for one hour at 37°C, resulting in <1% release of free measurable radio activity in the supernatant.

Endonuclease activity: 1µg of Type 1 supercoiled plasmid DNA is incubated with 500 units of enzyme in 1X reaction buffer for one hour at 37°C. The supercoiled DNA is visualized on an ethidium bromide stained agarose gel to verify absence of nicking or cutting activity.

Purity: One-Step RT-qPCR master mix is free of detectable RNase, and DNase (exo- and endonuclease) activity.

Stability and Storage

The One-Step RT-qPCR 2X master mix can be stored at +2°C to +8°C for about 4 weeks but should be stored at -20°C for long term storage to keep its excellent activity.

If kept at -20°C the kit is stable for at least 12 months.

When stored under these conditions and handled correctly, these products can be kept at least until the expiration date (see tube label) without showing any reduction in performance.

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).

This product does not require a Material Safety Data Sheet because it does neither contain more than 1% of a component classified as dangerous or hazardous nor more than 0.1% of a component classified as carcinogenic. However, we generally recommend, when working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

Genaxxon bioscience takes no liability for damage resulting from handling or contact with this product.

More information can be found in the REGULATION (EC) No. 1272/2008 OF THE EUROPEAN PARLIAMENT AND THE COUNCIL or contact Genaxxon bioscience (info@genaxxon.com).

Product Use Limitations

The Genaxxon biosience One-Step RT-qPCR 2X master mix 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.

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RT-qPCR Protocol Part

Important notes before getting started

- **Thaw One-Step RT-qPCR 2X master mix and primer solutions** on ice.
  Keep the solutions on ice after complete thawing.
  Mix well before use to avoid localized differences in salt concentration.

Recommended protocols for RT-qPCR:

Add the following components to a nuclease-free microtube. Pipette on ice and mix the components by pipetting gently up and down. In general, water, RNA and primers should be mixed together before the remaining components are added.

Component	Stock conc.	Final conc.,	20µL assay	50µL assay
RNA template	-	up to100ng polyA RNA or total RNA	x µL	x µL
forward Primer	10µM	400nM *	0.8µL	2µL
reverse Primer	10µM	400nM *	0.8µL	2µL
Dual-labelled Probe	10µM	200nM *	0.4µL	1µL
PCR-grade water	-	-	fill up to 20µL	fill up to 50µL
One-Step RT-qPCR 2X master mix **	2X	1X	10µL	25µL

* The optimal concentration for primers and probes may vary from 100nM to 500nM

** The One-Step RT-qPCR 2X master mix contains already RNase inhibitor that may be essential when working with low amounts of starting RNA.

Continue with the RT-qPCR protocol as recommended (see below).

Reverse transcription and thermal cycling

Place the vials of the RT-qPCR mix in a PCR cycler and start the following program.

Step	Temperature	Incubation	Repeats
1. Reverse transcription	50 to 55°C**	10-15 min. *	1x
2. Initial denaturation	95°C	5 min. ***	1x
3a. denaturation 3b. annealing 3c. elongation	95°C 60-65°C **** 72°C	15 sec 20 sec. 1 min/kb *****	35-45x

* A reverse transcription time of 10 min is recommended for optimal amplicon lengths between 100bp and 200bp. Longer amplicons up to 500 bp may require a prolonged incubation of 15 min.
** The optimal temperature depends on the structural features of the RNA. Increase the temperature to 55°C for difficult templates with high secondary structure. Note that optimal reaction time and temperature should be adjusted for each particular RNA.
*** An initial denaturation time of 5 min is recommended to inactivate the reverse transcriptase!
**** The annealing temperature depends on the melting temperature of the primers and DNA probe used.
***** The elongation time depends on the length of the amplicon.
A time of 1 min for a fragment of 1,000bp is recommended.

For optimal specificity and amplification an individual optimization of the recommended parameters may be necessary.

Note: the optimal reaction times and temperatures should be adjusted for each particular RNA / primer pair.