INSTRUCTIONS FOR USE

Rons Ultimate Green One-Step RT-qPCR Master Mix

List of Components	Quantity REF 125520
Rons Ultimate Green One-Step RT-qPCR Master Mix	1 ml (100 reactions)

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Introduction

Rons Ultimate Green One-Step RT-qPCR Master Mix is a ready-to-use 2x master mix for one-step reverse transcription and real-time PCR (RT-qPCR) with detection by a green fluorescent dye that binds double-stranded DNA. Reverse transcription and amplification are carried out consecutively in the same tube, so no separate cDNA synthesis is required. The mix contains a thermostable reverse transcriptase, a modified hot-start DNA polymerase, an RNase inhibitor, dNTPs and $MgCl_2$ in an optimized buffer system, together with the detection dye. Only primers, template RNA and water have to be added, which reduces pipetting steps and the risk of contamination.

The optimized reaction chemistry supports robust and efficient amplification across a broad range of RNA samples, including low-abundance targets and RNA templates with complex secondary structures. Incorporation of the green fluorescent DNA-binding dye enables real-time detection of amplified products and allows post-amplification melting curve analysis for assessment of amplicon specificity.

Applications

The master mix is intended for the detection and quantification of RNA targets in singleplex, dye-based RT-qPCR assays, for example:

- Gene expression analysis
- Detection of RNA viruses

The specificity of the amplification product can be verified by melt curve analysis. The mix is suitable for low amounts of template RNA and for RNA templates with complex secondary structures. Due to the short run time, it can also be used for high-throughput applications.

Principle of the method

In the first step, the reverse transcriptase synthesizes cDNA from the RNA template. A subsequent heating step inactivates the reverse transcriptase, and the cDNA is amplified by the hot-start DNA polymerase in a two-step cycling protocol consisting of denaturation and a combined annealing/extension step. The green dye fluoresces when bound to double-stranded DNA, so the signal recorded in each cycle increases with the amount of amplified product. As the dye binds to any double-stranded DNA, a melt curve analysis after amplification is recommended to distinguish the specific product from primer dimers and other non-specific products.

Product Specifications

Parameter	Specification
Concentration	2x
Detection chemistry	Green fluorescent dsDNA-binding dye
Assay format	One-step RT-qPCR, singleplex, dye-based
Enzymes	Thermostable reverse transcriptase, modified hot-start DNA polymerase, RNase inhibitor
Reaction volume	20 µl (10 µl reactions possible)
Template input	10 fg – 10 ng RNA per reaction
Reference dye	Not included (a low-ROX version available on request)

Product use limitations

- For research use only. Not for use in clinical or in vitro diagnostic procedures.
- The master mix is designed for single-plex detection with a DNA-binding dye. For probe-based multiplex assays, we recommend our Rons Ultimate One-Step RT-PCR Master Mix, REF 125610.
- For cDNA synthesis only, we recommend our Rons Ultimate cDNA Synthesis Kit, REF 125800.
- The master mix does not contain ROX. For real-time PCR instruments that require ROX as passive reference dye, a version with a low ROX concentration is available on request.

Warning and precautions

- Do not vortex the master mix, as this may damage the enzymes. Mix by gently inverting the tube.
- Store and handle the master mix protected from light.

Transportation & Storage

- Upon receipt, store the master mix at -22 °C to -18 °C.
- For short-term storage, the master mix can be kept at +2 °C to +8 °C for up to 1 month.
- Exposure to room temperature for a few hours up to 3 days does not affect the performance of the master mix.

Storage	Temperature and Conditions
Long Term	-22 °C to -18 °C
Short Term	+2 °C to +8 °C (up to 1 month)
Shelf Life	24 months from date of manufacture

Handling Recommendations / Procedure

Thaw the master mix, invert the tube 5 – 6 times and spin it down briefly. Reactions can be set up at room temperature. Pipette the components as listed below. For 10 µl reactions, use half of each volume.

Component	20 µl Reaction	Final Concentration
2x Master Mix	10 µl	1x
Forward primer (e.g. 10 µM)	variable (e.g. 0.8 µl)	0.1 – 0.4 µM
Reverse primer (e.g. 10 µM)	variable (e.g. 0.8 µl)	0.1 – 0.4 µM
Template RNA	variable	10 fg – 10 ng per reaction
PCR-grade water	adjust to 20 µl final volume	-

Mix gently by pipetting, centrifuge briefly and place the reactions in the real-time PCR instrument.

Recommended Thermocycler Protocol

Step	Time	Temperature	Cycles
Reverse transcription	10 minutes	50 – 55 °C	1 x
RT inactivation / initial denaturation	3 minutes	95 °C	1 x
Denaturation	5 seconds	92 °C	40 – 45 x
Annealing / extension	10 – 15 seconds	60 °C *	
Melt curve (optional) **	-	60 °C → 95 °C	-

***** Depending on the primers. For maximum efficiency and specificity, the annealing temperature, the annealing/extension time, the primer concentration and the amount of template RNA may need to be optimized. Cycling conditions may also have to be adapted to the instrument used.

** To verify the specificity of the amplification product, a melt curve can be recorded after the last cycle by increasing the temperature from 60 °C to 95 °C.

Symbol	Meaning	Symbol	Meaning
REF	Catalogue number	LOT	Batch code
	Temperature limit		Follow the instructions for use
	Manufacturer		Use-by date
	Date of manufacture	Σ	Contains sufficient for <n> tests
	Caution		Keep away from sunlight

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