INSTRUCTIONS FOR USE

Rons Ultimate HotRox Master Mix, Low ROX

List of Components	Quantity REF 128505
Rons Ultimate HotRox Master Mix, Low ROX (brown cap)	2 x 1 ml
$MgCl_2$, 100 mM (green cap)	1 ml
PCR Water (transparent cap)	2 x 1.8 ml

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Introduction

Rons Ultimate HotRox Master Mix, Low ROX is a ready-to-use 2x master mix for quantitative real-time PCR (qPCR) with probe-based detection. It contains a hot-start Taq DNA polymerase, dNTPs, $MgCl_2$ and reaction buffer as well as the passive reference dye ROX at a low concentration. Primers, probe(s), template DNA or cDNA and water are added by the user.

Applications

The master mix is intended for qPCR assays with sequence-specific fluorescent probes, such as hydrolysis probes, molecular beacons and MGB probes. Typical applications are:

- gene expression analysis with cDNA as template
- genotyping
- copy number analysis
- other probe-based quantitative PCR assays, including assays with low template amounts

Principle of the method

The probe binds to its target sequence between the two primers. During amplification, the fluorescence signal of the probe increases in proportion to the amount of PCR product and is measured in every cycle. The hot-start polymerase is inactive during reaction setup and is activated in the initial denaturation step.

ROX is used as passive reference dye. Its fluorescence does not change during the PCR run, so the instrument software can use it to normalize the reporter signal. This compensates for well-to-well differences caused, for example, by uneven illumination, small pipetting deviations or air bubbles, and improves the comparability of Cq values within and between runs. The 2x mix contains 100 nM ROX, which results in a final concentration of 50 nM in the reaction (Low ROX).

Product Specifications

Parameter	Specification
Format	2x concentrated, ready-to-use master mix
Polymerase	Hot-start Taq DNA polymerase
Detection	Fluorescent probes (hydrolysis probes, molecular beacons, MGB probes)
Passive reference dye	ROX, 100 nM in 2x mix (50 nM final concentration)
Template	DNA or cDNA, 1 pg – 10 ng per reaction
Reaction volume	20 µl (standard) or 10 µl
Number of reactions	200 reactions of 20 µl (2 x 1 ml master mix)

Product use limitations

- For research use only. Not intended for diagnostic or clinical procedures.
- Check before use whether the real-time PCR instrument requires a passive reference dye and at which concentration.
- Primers, probe(s) and template are not included and have to be provided by the user.
- The mix is designed for probe-based detection. Rons Ultimate GreenHot Master Mix, Low ROX, a master mix for detection with green fluorescent dye is available under REF 128705.

Warning and precautions

- Do not vortex the master mix, as this may damage the enzyme. Mix by gently inverting the tube.
- Do not keep the master mix at room temperature for longer than 3 days.

Transportation & Storage

- Store all components at -22 °C to -18 °C upon receipt.
- Keeping the master mix at room temperature for a few hours up to 3 days does not affect its performance.

Storage	Temperature and Conditions
Long Term	-22 °C to -18 °C
Short Term	+2 °C to +8 °C, up to 1 month
Room Temperature	up to 3 days
Shelf Life	24 months from date of manufacture (stored at -22 °C to -18 °C)

Handling Recommendations / Procedure

Thaw the master mix completely, mix by inverting the tube 5–6 times and centrifuge briefly. Reactions can be set up at room temperature.

The pipetting scheme below refers to a 20 µl reaction. For 10 µl reactions, use half of each volume; the final concentrations remain the same.

Component	20 µl Reaction	Final Concentration
2x Master Mix	10 µl	1x
Forward Primer	variable	0.1 – 0.4 µM
Reverse Primer	variable	0.1 – 0.4 µM
Probe	variable	0.2 – 0.4 µM
Template DNA / cDNA	variable	1 pg – 10 ng per reaction
PCR Water	adjust to 20 µl final volume	-

Recommended Thermocycler Protocols

Either of the following protocols can be used. In the 2-step protocol, annealing and extension are combined in one step.

3-step protocol

Step	Time	Temperature	Cycles
Initial Denaturation	2 minutes	92 – 95 °C	1 x
Denaturation	5 seconds	92 – 95 °C	30 – 45 x
Annealing	5 seconds	60 °C*	
Extension	5 – 10 seconds	72 °C	

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2-step protocol

Step	Time	Temperature	Cycles
Initial Denaturation	2 minutes	92 – 95 °C	1 x
Denaturation	5 seconds	92 – 95 °C	30 – 45 x
Annealing / Extension	5 – 15 seconds	60 °C*	

***** 60 °C is a recommended starting value. The optimal annealing temperature depends on the primers and probe used.

For maximum efficiency and specificity, annealing temperature, extension time, primer and probe concentrations and template amount may need to be optimized.

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

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