INSTRUCTIONS FOR USE

Rons Ultimate GreenHot Master Mix, Low ROX

List of Components	Quantity REF 128705
Rons Ultimate GreenHot Master Mix, Low ROX (brown cap)	2 x 1 ml (2 x 100 reactions)
$MgCl_2$, 100 mM (green cap)	1 ml
PCR water (transparent cap)	2 x 1.8 ml

IFU valid from	**November 2026**
IFU reviewed	**November 2026**

BIORON GmbH – In den Rauhweiden 20 – 67354 Römerberg - Germany
Phone:+49623229845-0 - Fax:+49623229845-29
info@bioron.net www.bioron.net

Introduction

Rons Ultimate GreenHot Master Mix, Low ROX is a ready-to-use, 2x concentrated master mix for real-time PCR (qPCR) with detection by a green fluorescent dye. It contains a hot-start Taq DNA polymerase, dNTPs, $MgCl_2$, a green fluorescent dye and the passive reference dye ROX in a reaction buffer optimized for qPCR. Only primers, template and PCR water have to be added, which reduces pipetting steps and the risk of contamination.

Applications

The master mix is intended for quantitative real-time PCR with DNA or cDNA as template, for example:

- gene expression analysis (cDNA),
- genotyping,
- copy number analysis,
- other quantitative PCR assays.

The master mix supports amplification from low template amounts as well as from templates that are difficult to amplify.

Principle of the method

The green fluorescent dye binds to double-stranded DNA, which strongly increases its fluorescence. As PCR product accumulates, the fluorescence signal increases accordingly and is measured by the real-time PCR instrument in every cycle. The hot-start Taq DNA polymerase is inactive during reaction setup and is activated by 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	Green fluorescent DNA-binding dye
Passive reference dye	ROX, 100 nM in the 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 and template are not included and have to be provided by the user.
- For a dye-based qPCR master mix without ROX dye, Rons Ultimate GreenHot Master Mix (REF 128205) is available.
- For a probe-based qPCR Master Mix with ROX, please order Rons Ultimate HotRox Master Mix, REF 128505.

Warning and precautions

- Do not vortex the master mix, to avoid damaging 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 at -22 °C to -18 °C upon receipt.
- Storage at 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 (stored at -22 °C to -18 °C)

Handling Recommendations / Procedure

Thaw the master mix completely, mix by gently inverting the tube 5–6 times (do not vortex) 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
Template DNA / cDNA	variable	1 pg – 10 ng per reaction
PCR Water	adjust to 20 µl final volume	-

BIORON GmbH – In den Rauhweiden 20 – 67354 Römerberg - Germany
Phone:+49623229845-0 - Fax:+49623229845-29
info@bioron.net www.bioron.net

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	

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 *	

***** The optimal annealing temperature depends on the primers used; 60 °C is a suitable starting point.

To obtain the best efficiency and specificity, annealing temperature, extension time, primer concentration and template amount may have to be optimized for each assay.

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

BIORON GmbH – In den Rauhweiden 20 – 67354 Römerberg - Germany
Phone:+49623229845-0 - Fax:+49623229845-29
info@bioron.net www.bioron.net