



One-Step qRT-PCR Master Mix, Low ROX (2X)

Catalog No. 17-2003-50: One-Step qRT-PCR Master Mix, Low ROX (2X), Size: 50 reactions (1.25 ml)
Catalog No. 17-2003-200: One-Step qRT-PCR Master Mix, Low ROX (2X), Size: 200 reactions (4X1.25 ml)
Catalog No. 17-2003-500: One-Step qRT-PCR Master Mix, Low ROX (2X), Size: 500 reactions (10X1.25 ml)

Catalog No. 17-2004-50: One-Step qRT-PCR Master Mix, (2X), Size: 50 reactions (1.25 ml)
Catalog No. 17-2004-200: One-Step qRT-PCR Master Mix, (2X), Size: 200 reactions (4X1.25 ml)
Catalog No. 17-2004-500: One-Step qRT-PCR Master Mix, (2X), Size: 500 reactions (10X1.25 ml)

Storage:

ABEOMICS One-Step qRT-PCR Master Mix, Low Rox is stable for 1 year when stored at -20°C. No significant performance loss was detected after 10 times freeze-thaw cycles. For convenience, it may be stored at 4°C for up to two weeks.

Description:

ABEOMICS One-Step qRT-PCR Master Mix, Low Rox is a 2X concentrated formulation mix. It is designed for the reverse transcription (RT) and polymerase chain reaction (PCR) amplification of a specific target RNA from either total RNA or mRNA in a single tube for the detection and quantification of RNA using real-time detection instruments. The proprietary 2X qRT-PCR Master Mix contains an engineered Script Reverse Transcriptase (RNaseH minus), Hot Start *Taq* DNA polymerase, dNTPs, Mg²⁺, enhancer, stabilizer and an optimal concentration of ROX for instruments that require low ROX for a reference signal. Please consult our website and Instrument Compatibility section below for the correct product for your real-time PCR system.

The Hot Start *Taq* DNA polymerase is an antibody-inactivated hot-start enzyme designed to block polymerase activity between ambient to RT reaction temperature. Script RT enzyme can synthesize cDNA at a temperature range of 40-55°C. Once the PCR step reaches denaturation temperature (95°C), *Taq* DNA polymerase activity is restored and the resulting PCR exhibits higher sensitivity, specificity and yield.

The system enables highly sensitive detection from as few as 1 copy of a target gene, with a broad dynamic range that supports accurate quantification of high-copy mRNA from up to 1 µg of total RNA. Sufficient reagents are provided for 500 amplification reactions of 50 µl each or 1250 amplification reactions of 20 µl each.

Features:

- Convenient and easy-to-use one tube 2X Master Mix for systems that require low ROX
- Broad range of RT reaction temperature (40-55°C)
- Broad dynamic range of detection
- Hot start PCR system
- Fully optimized qRT-PCR buffer and robustness
- High sensitivity, specificity, and reproducibility
- Ideal for TaqMan probe based everyday qRT-PCR

Product qualification

ABEOMICS One-Step qRT-PCR Master Mix, Low Rox demonstrates high RT-qPCR efficiency and linear resolution over a wide linear dynamic range. Stringent specifications are maintained to ensure lot to lot consistency. This product is free of detectable DNase and RNase activities.

Kit components:

Catalog No.	Volume	Total Reaction
17-2003-50	1.25 ml	50 nos
17-2003-200	4 X 1.25 ml	200 nos
17-2003-500	10 X 1.25 ml	500 nos

ABEOMICS One-Step qRT-PCR Master Mix, Low Rox (Contains t RT, Hot Start Taq, dNTPs, 6 mM Mg²⁺, enhancers and stabilizers)

Recommended qRT-PCR reaction assembly:

The following protocol is suggested as a starting point. For multiple reactions, prepare a master mix of common components by adding the appropriate volume to each tube or plate well on ice, and then add the unique reaction components (e.g., RNA template).

1. Thaw all components at room temperature. Hand warming can facilitate the process. Mix gently, then centrifuge to collect contents to the bottom of the tube before using. Place all components on ice after thawing.
2. Assemble the reaction on ice.

Components	50 µl Rxn	20 µl Rxn	Final Concentration
ABEOMICS One-Step qRT-PCR Master Mix, Low Rox (2X)	25 µl	10 µl	1X
Forward Primer (10 µM)	1.0 µl	0.4 µl	200nM (Variable 100-900 nM)
Reverse Primer (10 µM)	1.0 µl	0.4 µl	200nM (Variable 100-900 nM)
RNA Template	x µl	x µl	Variable (0.1 pg - 1 µg)
Fluorogenic Probe (10µM)	0.5 µl	0.2 µl	100nM (Variable 100-250 nM)
Volume with distilled water	to 50 µl	to 20 µl	

Note: Reaction volume can be scaled from 5 - 50 µl depending on the reaction plate (i.e. 384-well vs. 96-well) and qRT-PCR system. Scale all component volumes proportionally.

3. After capping each reaction, vortex gently to mix contents, centrifuge briefly to collect components at the bottom of the reaction tube. The assembled reactions should be kept cold to maximize the sensitivity and specificity of the assay until placed in real-time PCR system.
4. Program the thermal cycler so that cDNA synthesis is followed immediately by PCR amplification.

cDNA Synthesis RT inactivation & Taq activation PCR cycling (30-45 cycles)	Standard Cycling	Fast Cycling
	50°C, 10 min	50°C, 5 min
	95°C, 2 min	95°C, 2 min
	95°C, 10-15s	95°C, 3-5s
	60°C, 30-60s	60°C, 20-30s
	(Data collection)	(Data collection)

Optimal cycling conditions will vary for different primer sets.

Melting curve analysis (Refer to instrument documentation).

Note: Efficient cDNA synthesis can be achieved between 3-15 minutes of incubation at 45-55°C. We recommend 5 minutes of incubation at 50°C as a general starting point.

5. Place reactions in a preheated real-time instrument programmed as described above. Collect data and analyze results.

Recommendations and Guidelines for One Step qRT-PCR

Instrument Compatibility

This kit can be used with a variety of real-time instruments, including but not limited to the Bio-Rad CFX96 Touch, CFX384 Touch, CFX Connect, the Corbett Research Rotor-Gene Q, Rotor-Gene 3000, Rotor-Gene 6000; Illumina Eco qPCR etc.

Reagent	Catalog Number	Compatible Real-Time PCR System
ABEOMICS One-Step qRT-PCR Master Mix	17-2004-05 17-2004-20 17-2004-50	Bio-Rad CFX96, CFX384, CFX Connect; the Corbett Research Rotor-Gene Q, Rotor-Gene 3000, Rotor-Gene 6000; Illumina Eco qPCR; Roche LightCycler™ 480; Eppendorf Mastercycler® ep realplex
ABEOMICS One-Step qRT-PCR Master Mix, Low Rox	17-2003-05 17-2003-20 17-2003-50	ABI® 7500, 7500 Fast, ViiA 7 Real-Time PCR Systems, the Stratagene Mx3000P®, Mx3005P™, and Mx4000®; the Agilent AriaMx

Template

Starting material can range from 1 pg to 1 µg of purified total RNA. RNA should be free of RNase contamination and aseptic conditions should be maintained. RNA may be treated with DNase I to remove any contaminating DNA.

Magnesium Concentration

The 2X Reaction Mix includes magnesium at a final concentration of 3 mM. This works well for most targets; however, the optimal concentration may range from 3 to 6 mM. If necessary, use the following table to determine the amount of MgCl₂ to add to achieve the specified concentration:

For a Final MgCl ₂ Concentration of	Add this Volume of 50 mM MgCl ₂ (per 50 µl Rxn)
4.0 mM	1 µl
5.0 mM	2 µl
6.0 mM	3 µl

Melting Curve Analysis:

Melting curve analysis should always be performed during qRT-PCR to identify the presence of primer dimers and analyze the specificity of the reaction.

Primers:

Gene-specific primers are required. The length of amplicon should be approximately 70–150 bp, and the primers should be designed to anneal to exons on both sides of an intron or within the exon/exon boundary of the mRNA to allow differentiation of cDNA from genomic DNA. A final concentration of 200 nM per primer is effective for most reactions. Optimal results may require a titration of primer concentrations between 200 and 900 nM.

Troubleshooting Guide:

Problem	Possible Cause	Solution
Poor sensitivity	Not enough template RNA	Increase concentration of template RNA; use 10 ng–1 µg total RNA.
Product detected at higher than expected cycle number	RNA has been damaged/degraded RNase contamination Inefficient cDNA synthesis Inefficient PCR amplification	Replace RNA if necessary. Maintain aseptic conditions; add RNase inhibitor. Adjust cDNA synthesis temperature and/or primer design. Double the amount of reverse primer (e.g., to 400 nM). Optimize PCR conditions: Adjust annealing temperature as necessary. Increase magnesium concentration.
Higher than expected signal	Too much sample added to reactions	Decrease the concentration of template RNA.
Product detected at lower-than-expected cycle number, and/or positive signal from no-template controls	Template or PCR carry-over contamination	Isolate source of contamination and replace reagent(s). Use separate dedicated pipettors for reaction assembly and post-PCR analysis. Assemble reactions (except for target addition) in a DNA-free area. Use aerosol-resistant pipet tips or positive displacement pipettors.
Unexpected bands after electrophoresis	Genomic DNA contamination Oligo(dT) or random primers used for cDNA synthesis	Pre-treat RNA with DNase I. Use only gene-specific primers.

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