Lyo-Ready 1-Step RT-qPCR ROX Mix

Product Handling Guide

Shipping:	Blue Ice
Catalog number:	MDX174
Batch No.:	See vial
Concentration:	4x

Store at –20 °C

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Storage and stability:
Lyo-Ready 1-Step RT-qPCR ROX Mix is shipped on blue ice. On arrival store at -20 °C for optimum stability. Repeated freeze/thaw cycles should be avoided. Thawing during transportation does not affect the product performance. Solutions should be mixed/equilibrated after each thawing to avoid phasing.

Safety precautions:
Read and understand the SDS (Safety Data Sheets) before handling the reagents. The SDS (Safety Data Sheets) are available upon request.

Quality control:
Meridian operates under ISO 13485 Quality Management System. Lyo-Ready 1-Step RT-qPCR ROX Mix is tested functionally before its release (see Test Release document)

Notes:
For research and further manufacturing use only.

Description

Lyo-Ready 1-Step RT-qPCR ROX Mix is a glycerol-free, one tube formulation combining the latest advances in buffer chemistry and PCR enhancers, together with an optimized concentration of reverse transcriptase, antibody-mediated hot-start polymerase, dNTPs and $MgCl_2$. Lyo-Ready 1-Step RT-qPCR ROX Mix has been designed for highly reproducible, accurate RNA and DNA target amplification, delivering excellent results in multiplex assays. In order to produce room temperature lyophilized RT-qPCR reagents, assay specific primers and probes can be added to Lyo-Ready 1-Step RT-qPCR ROX Mix for subsequent lyophilization.

Kit components

Table 1

Component
Lyo-Ready 1-Step RT-qPCR ROX Mix, 4x

Users Guidelines

Master mix preparation

Recommended reagent volumes per 20 µL RT-qPCR mix are given in Table 2.

Table 2

Reagent	Volume
Lyo-Ready 1-Step RT-qPCR ROX Mix, 4x	5 µL
Primer-Probe Mix, 20x	1 µL*
Water	x µL
Total volume	Up to 20 µL

** Primer and probe concentration needs be optimized*

Dispense into reaction vials and immediately transfer into a freeze-dryer and run a suitable drying cycle.

For long-term storage at ambient temperatures, the lyophilized product from the freeze-dryer should be packaged with a silica sachet in a heat sealed pouch at low relative humidity conditions.

Assay setup

Rehydrate the lyophilized RT-qPCR master mix in the reaction vials with 20 µL template-containing solution and run RT-qPCR.

The RT-qPCR conditions in Table 3 are suitable for amplicons of up to 200 bp. These cycling parameters have been optimized for Lyo-Ready 1-Step RT-qPCR ROX Mix on a number of platforms, however they can be varied to suit different machine-specific protocols.

Table 3

Step	Temperature	Time	Cycles
Reverse transcription	50 °C	10 mins	1
Polymerase activation	95 °C	2 min	1
Denaturation	95 °C	10 s	45
Annealing/Extension*	60 °C	30 s	

- *When multiplexing, the annealing/extension time can be extended up to 60 seconds and/or the annealing/extension temperature can be increased up to 65°C.*

Optional ROX: Lyo-Ready 1-Step RT-qPCR ROX Mix is premixed with ROX (5-carboxy-X-rhodamine, succinymidyl ester), so that where necessary, ROX fluorescence can be optionally detected on certain real-time instruments. If your real-time instrument has the capability of using ROX and you wish to use this option, then this option must be selected by the user in the software

Technical Support

For any technical enquiries, please contact our Technical Support team via email at: mbi.tech@meridianlifescience.com

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