

Inhibitor-Tolerant RT-qPCR Mix

Product Handling Guide

| | |
|-----------------|-----------------|
| Shipping: | On Dry/Blue Ice |
| Catalog number: | MDX016 |
| Batch No.: | See vial |
| Concentration: | 4x |

Store at -20 °C



Storage and stability:

Inhibitor-Tolerant RT-qPCR Mix is shipped on dry/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.

Expiry:

When stored under the recommended conditions and handled correctly, full activity of the kit is retained until the expiry date on the outer box label.

Safety precautions:

Read and understand the SDS (Safety Data Sheets) before handling the reagents. Hardcopies of the SDS will be provided with the first shipment, thereafter they will be available upon request.

Quality control:

Bioline operates under ISO 13485 Quality Management System. Inhibitor-Tolerant RT-qPCR Mix and its components are extensively tested for activity, processivity, efficiency, heat activation sensitivity, absence of nuclease contamination and absence of nucleic acid contamination.

Notes:

This reagent has been manufactured under 13485 Quality Management System and is suitable for further manufacturing use as an IVD component.

Description

Inhibitor-Tolerant RT-qPCR Mix is a one tube formulation combining the latest advances in buffer chemistry and PCR enhancers, together with an optimized concentration of antibody-mediated hot-start polymerase, reverse transcriptase, RNase Inhibitors, dNTPs and MgCl₂. Inhibitor-Tolerant RT-qPCR Mix has been designed for highly reproducible, accurate RNA and DNA target amplification under fast thermal cycling conditions, delivering excellent results in multiplex assays, even in the presence of difficult inhibitors found in sputum and stool samples.

Kit components

Table 1

| Component |
|------------------------------------|
| Inhibitor-Tolerant RT-qPCR Mix, 4x |

Users Guidelines

Master mix preparation

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

Table 2

| Reagent | Volume |
|------------------------------------|--------------------|
| Inhibitor-Tolerant RT-qPCR Mix, 4x | 5 µL |
| Primer-Probe Mix, 20x | 1 µL |
| Template* | Up to 10 µL |
| Water | As required |
| Total volume | Up to 20 µL |

*RNA and DNA template from extracted or crude sample lysate. Template volume optimization required.

Assay setup

The qPCR conditions in Table 3 are suitable for amplicons of up to 200 bp. These cycling parameters have been optimized for Inhibitor-Tolerant RT-qPCR 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 min | 1 |
| Polymerase activation | 95 °C | 2 min | 1 |
| Denaturation | 95 °C | 5 s | 45 |
| Annealing/Extension** | 60 °C | 20 s | |

**When multiplexing, the reverse transcription reaction time can be extended up to 20 minutes and/or the temperature can be increased up to 55° C and the annealing/extension time can be extended up to 60 seconds and/or the temperature can be increased up to 65° C.

Associated products

| Product | Cat. No. |
|------------------------------|----------|
| Inhibitor-Tolerant qPCR Mix | MDX013 |
| Fast 1-Step RT-qPCR Mix | MDX032 |
| Lyo-Ready 1-Step RT-qPCR Mix | MDX024 |

Technical Support

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