Lyo-Ready™ Inhibitor-Tolerant qPCR Mix Product Handling Guide		Storage and stability: Lyo-Ready™ Inhibitor-Tolerant qPCR 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.
		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.
Shipping:	Blue Ice	Safety precautions:
Catalog number:	MDX184	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.
Batch No.:	See vial	Quality control:
Concentration:	4x Store at −20 °C	Meridian operates under ISO 13485 Quality Management System. Lyo-Ready™ Inhibitor-Tolerant 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.
		<b>Notes:</b> For research or further manufactured use only.

## Description

Lyo-Ready<sup>™</sup> Inhibitor-Tolerant qPCR Mix is a glycerol-free, one tube formulation combining the latest advances in buffer chemistry and PCR enhancers, together with an optimized concentration of antibody-mediated hot-start polymerase, dNTPs and MgCl<sub>2</sub>. Lyo-Ready<sup>™</sup> Inhibitor-Tolerant qPCR Mix has been designed for highly reproducible, accurate DNA target amplification, delivering excellent results in multiplex assays, even in the presence of difficult inhibitors found in blood, sputum or stool samples. In order to produce room temperature lyophilized qPCR reagents, assay specific primers and probes can be added to Lyo-Ready<sup>™</sup> Inhibitor-Tolerant qPCR Mix for subsequent lyophilization.

#### Kit components

#### Table 1

#### Component

Lyo-Ready™ Inhibitor-Tolerant qPCR Mix, 4x

# **Users Guidelines**

## Master mix preparation

Recommended reagent volumes per 20  $\mu L$  qPCR mix are given in Table 2.

## Table 2

Reagent	Volume
Lyo-Ready™ Inhibitor-Tolerant qPCR Mix, 4x	5 µL
Primer-Probe Mix, 20x	1 µL*
Water	xμL
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 qPCR master mix in the reaction vials with 20  $\mu L$  template-containing solution and run qPCR.

The qPCR conditions in Table 3 are suitable for amplicons of up to 200 bp. These cycling parameters have been optimized for Lyo-Ready<sup>TM</sup> Inhibitor-Tolerant qPCR Mix on a number of platforms, however they can be varied to suit different machine-specific protocols.

#### Table 3

Step	Temperature	Time	Cycles	
Polymerase activation	95 °C	2 min	1	
Denaturation	95 °C	5 s	45	
Annealing/Extension*	60 °C	20 s	- 45	

\* 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.

#### **Technical Support**

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