Lyo-Ready <sup>™</sup> Direct RNA/DNA qPCR Stool Product Handling Guide		Storage and stability: Lyo-Ready™ Direct RNA/DNA qPCR Stool is shipped 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.	
Batch No.: Concentration:	See vial 4x Store at –20 °C	Quality control: Meridian Bioscience operates under ISO 13485 Quality Management System. Lyo-Ready™ Direct RNA/DNA qPCR Stool and its components are extensively tested for activity, processivity, efficiency, heat activation sensitivity, absence of nuclease contamination and absence of nucleic acid contamination.	
		Notes: For research or further manufactured use only.	

## Description

Lyo-Ready<sup>™</sup> Direct RNA/DNA qPCR Stool 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, reverse transcriptase, RNase Inhibitor, dNTPs and MgCl<sub>2</sub>. Lyo-Ready<sup>™</sup> Direct RNA/DNA qPCR Stool has been designed for highly reproducible, accurate RNA and DNA target amplification, delivering excellent results in multiplex assays, even in the presence of crude respiratory tract samples. In order to produce room temperature lyophilized RT-qPCR reagents, assay specific primers and probes can be added to Lyo-Ready<sup>™</sup> Direct RNA/DNA qPCR Stool for subsequent lyophilization.

## **Kit components**

### Table 1

### Component

Lyo-Ready™ Direct RNA/DNA qPCR Stool, 4x

# **Users Guidelines**

## Master mix preparation

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

#### Table 2

Reagent	Volume
Lyo-Ready™ Direct RNA/DNA qPCR Stool, 4x	5 µL
Primer-Probe Mix, 20x	1 µL*
Total volume	6 µL

\* Primer and probe concentration need be optimized

Dispense into reaction vessels, immediately transfer into a freeze-drier and run a suitable drying cycle.

For long-term storage at ambient temperatures, the lyophilized product from the freeze-dryer should be packaged with silica desiccant 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  $\mu L$  template-containing solution, vortex 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<sup>™</sup> Direct RNA/DNA qPCR Stool on a number of platforms, however they can be varied to suit different machine-specific protocols.

### Table 3

Step	Temperature	Time	Cycles	
Reverse transcription*	45 °C - 50 °C	10 min	1	
Polymerase activation	95 °C	2 min	1	
Denaturation	95 °C	5 s	45	
Annealing/Extension*	60 °C	20 s	45	

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

## Associated products

Lyo-Ready™ Direct DNA qPCR Stool, 4x	MDX123
Lyo-Ready™ Direct RNA/DNA qPCR Blood, 4x	MDX123
Lyo-Ready™ Direct RNA/DNA qPCR Saliva, 4x	MDX133
Air-Dryable™ Direct DNA qPCR Stool, 4x	MDX140
Air-Dryable™ Direct RNA/DNA qPCR Stool, 4x	MDX141
Air-Dryable™ Direct RNA/DNA qPCR Blood, 4x	MDX121
Air-Dryable™ Direct RNA/DNA qPCR Saliva, 4x	MDX131

## **Technical Support**

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

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