# Lyo-Ready™ AOF Direct RNA/DNA qPCR Urine Product Handling Guide

Shipping: Blue Ice
Catalog number: MDX353

Batch No: See vial

Store at -20 °C



4x

#### Storage and stability:

Lyo-Ready<sup>TM</sup> AOF Direct RNA/DNA qPCR Urine 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.

#### 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:

Meridian operates under ISO 13485 Quality Management System. Lyo-Ready™ AOF Direct RNA/ DNA qPCR Urine and its components are extensively tested for activity and sensitivity.

#### Notes:

For research and further manufacturing use only.

## Description

Concentration:

Lyo-Ready™ AOF Direct RNA/DNA qPCR Urine 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, dNTP/dUTP mix and MgCl₂. Lyo-Ready™ AOF Direct RNA/DNA qPCR Urine has been designed for highly reproducible, accurate RNA and DNA target amplification, delivering excellent results in multiplex assays, even in the presence of urine samples. In order to produce room temperature lyophilized RT-qPCR reagents, assay specific primers and probes can be added to Lyo-Ready™ AOF Direct RNA/DNA qPCR Urine for subsequent lyophilization.

The mix is suitable for high-throughput assays where there is a greater risk of false positives from cross-over contamination.

#### Kit components

#### Table 1

#### Component

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

## **Users Guidelines**

## Master mix preparation for lyophilization

Recommended reagent volumes for the preparation of master mix per 20  $\mu$ L RT-qPCR reaction are given in Table 2.

#### Table 2

Reagent	Volume
Lyo-Ready™ AOF Direct RNA/DNA qPCR Urine, 4x	5 μL
Primer-Probe Mix, 20x*	1 μL
Water	Up to 10 μL

<sup>\*</sup> Primer and probe concentration must be optimized

<u>For preparation of lyo-cakes</u>, dispense into reaction vials, such as PCR strip tubes or plates, and immediately transfer into a freeze-dryer and run a suitable lyophilization cycle (See: Lyophilization and Post-lyophilization User Guideline).

<u>For preparation of lyo-beads</u>, dispense the liquid master mix into liquid nitrogen and immediately transfer into a freeze-dryer and run a suitable drying cycle (See: Lyo-bead production and Post-lyophilization User Guideline).

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  $\mu L$  template-containing solution and run the RT-qPCR reaction.

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™ AOF Direct RNA/DNA qPCR Urine 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	40

<sup>\*</sup> When multiplexing, the reverse transcription reaction time can be extended up to 20 minutes an 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**

Component	Cat. No.
Lyo-Ready™ AOF Direct DNA qPCR Urine, 4x	MDX352
Lyo-Ready™ AOF Direct DNA qPCR Blood, 4x	MDX322
Lyo-Ready™ AOF Direct RNA/DNA qPCR Blood, 4x	MDX323
Lyo-Ready™ AOF Direct DNA qPCR Saliva, 4x	MDX332
Lyo-Ready™ AOF Direct RNA/DNA qPCR Saliva, 4x	MDX333