| Lyo-Ready [™] Direct RNA/DNA qPCR BlooProduct Handling Guide | | Storage and stability: Lyo-Ready [™] Direct RNA/DNA qPCR Blood 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. | |
|--|-----------------------|--|--|
| | | | |
| Catalog number: Batch No.: | MDX123 See vial | 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. | |
| Concentration: | 4x Store at –20 °C | Quality control: Meridian operates under ISO 13485 Quality Management System. Lyo-Ready™ Direct RNA/DNA qPCR Blood and its components are extensively tested for activity, processivity, efficiency, heat activation sensitivity, absence of nuclease contamination and absence of nucleic acid contamination. | |
| merid | | Notes: For research or further manufactured use only. | |

Description

Lyo-Ready[™] Direct RNA/DNA qPCR Blood 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 MgCl₂. Lyo-Ready[™] Direct RNA/DNA qPCR Blood 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[™] Direct RNA/DNA qPCR Blood for subsequent lyophilization.

Kit components

Table 1

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

Users Guidelines

The amount of inhibition tolerated by Lyo-Ready[™] Direct RNA/DNA qPCR Blood is variable depending on several factors, including assay design and sample quality. For this reason, an initial sample titration is recommended.

Master mix preparation

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

Table 2

| Reagent | Volume |
|--|--------|
| Lyo-Ready™ Direct RNA/DNA qPCR Blood, 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 μL 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 are compatible with Lyo-Ready™ Direct RNA/DNA qPCR Blood on a number of platforms, however they can be varied to suit different detection assay and 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 | |

* 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

| Component | Cat. No. |
|--|----------|
| Air-Dryable™ Direct DNA qPCR Blood | MDX092 |
| Air-Dryable™ Direct RNA/DNA qPCR Blood | MDX121 |
| Lyo-Ready™ Direct DNA qPCR Blood | MDX122 |
| Lyo-Ready™ Direct DNA qPCR Saliva | MDX132 |
| Lyo-Ready™ Direct RNA/DNA qPCR Saliva | MDX133 |

Technical Support

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