

Lyo-Ready Direct Blood Mixes

Ultra-sensitive master mixes designed for direct qPCR/RT-qPCR from whole blood, serum or plasma samples.

Lyo-Ready™ Direct DNA qPCR Blood and Lyo-Ready™ Direct RNA/DNA qPCR Blood are 4x concentrated, glycerol-free mixes that are compatible with lyophilization and designed for ultra-sensitive amplification of RNA or DNA from crudely processed blood, serum or plasma.

Blood is one of the most common specimens used for laboratory diagnostic testing and it is useful for evaluating the function of vital organs (kidneys, liver, thyroid and heart) and for diagnosing diseases including bacterial and viral infections, cancer, cardiovascular disease, and metabolic disorders such as diabetes. However, there are several PCR inhibitors such as immunoglobulin G, hemoglobin, lactoferrin and leukocyte DNA that are present in blood which impact a reaction's efficiency. In addition, anticoagulants used to stabilize blood samples (e.g. EDTA, citrate or heparin) contain a number of inhibitors which cause interference in a PCR reaction. Traditional approaches to overcoming inhibition have relied on removing inhibitors through DNA or RNA extraction prior to testing. However, these methods are not 100% effective, cause sample loss, and reduce reproducibility.

Meridian's new Lyo-Ready™ direct blood mixes are unique by enabling direct amplification of target DNA or RNA from crudely processed blood, serum or plasma samples. They have been designed to efficiently amplify in the presence of inhibitors found in blood – no further optimization is required. Furthermore, these mixes can be used in a wet format or lyophilized to create ambient-temperature stable assays.

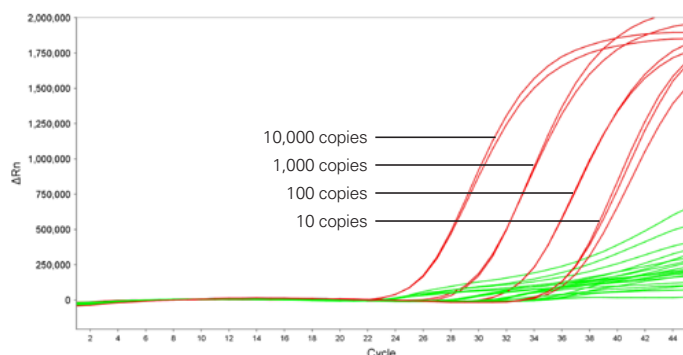
Product Highlights

- Ultra-sensitive detection down to less than 10 copies of DNA or RNA using direct amplification protocols
- Inhibitor-tolerant to PCR inhibitors found in whole blood, serum, plasma (20%) and anticoagulants (e.g. 20% EDTA)
- Suitable for singleplex or multiplex assays
- Mixes can be used as a liquid or lyophilized to extend assay shelf-life to +18 months
- Compatible with a range of lyophilization protocols
- Validated for detection of blood pathogens and liquid biopsy applications

| PRODUCT | CAT NO. | VOLUME | REACTIONS |
|--------------------------------------|---------|--------|-------------|
| Lyo-Ready™ Direct DNA qPCR Blood | MDX122 | 5 mL | 1,000 Rxns |
| | | 50 mL | 10,000 Rxns |
| Lyo-Ready™ Direct RNA/DNA qPCR Blood | MDX123 | 5 mL | 1,000 Rxns |
| | | 50 mL | 10,000 Rxns |

Ultra-sensitive detection of DNA or RNA from complex, inhibitor-rich blood samples

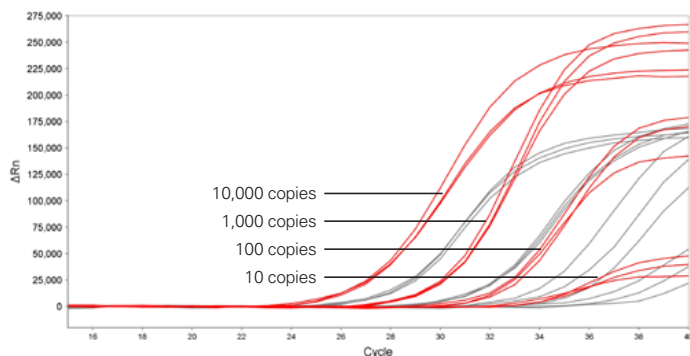
Sensitivity of DNA Amplification in the Presence of 20% Serum



Activity of lyophilized Lyo-Ready™ Direct DNA qPCR Blood (red) was compared to KAPA Probe Force (green) in a multiplex qPCR assay, using a 10-fold serial dilution of plasmid DNA (10,000, 1000, 100 and 10 copies respectively), in the presence of 20% serum. The results illustrate that Lyo-Ready™ Direct DNA qPCR Blood has significantly higher sensitivity and reproducibility than KAPA Probe Force in assays detecting high or low copies of DNA template and in the presence of PCR inhibitors.

Lyo-Ready™ Direct DNA qPCR Blood | KAPA Probe Force

Sensitivity of RNA Amplification in the Presence of 5% K2-EDTA Whole Blood

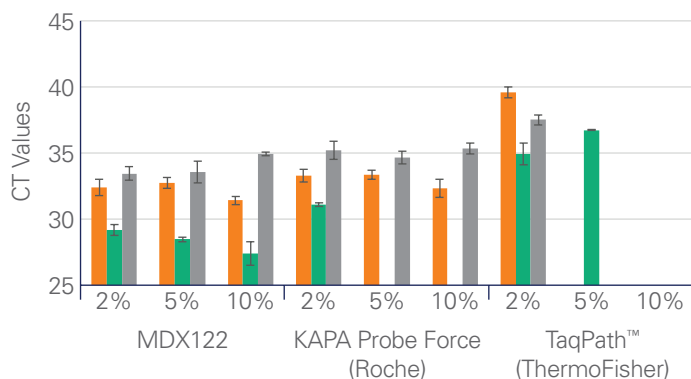


Lyo-Ready™ Direct RNA/DNA qPCR Blood (red) was compared to UltraPlex 1-Step ToughMix (grey) in a multiplex qPCR assay, using a 10-fold serial dilution of mammalian RNA (10,000, 1000, 100 and 10 copies respectively), in the presence of 5% K2-EDTA whole blood. The results illustrate that Lyo-Ready™ Direct RNA/DNA qPCR Blood has significantly higher sensitivity and reproducibility than UltraPlex in assays detecting high or low copies of RNA template and in the presence of PCR inhibitors.

Lyo-Ready™ Direct RNA/DNA qPCR Blood | UltraPlex 1-Step ToughMix

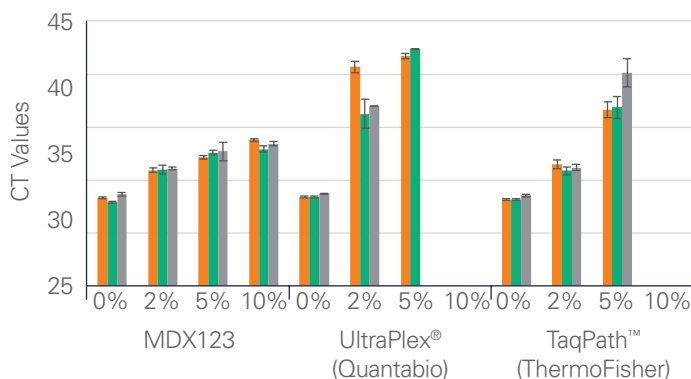
Significantly greater tolerance to inhibitors found in whole blood and anticoagulants (K2-EDTA, sodium heparin, and sodium citrate)

A/ Lyo-Ready™ Direct DNA qPCR Blood (MDX122)



EDTA | Heparin | Citrate

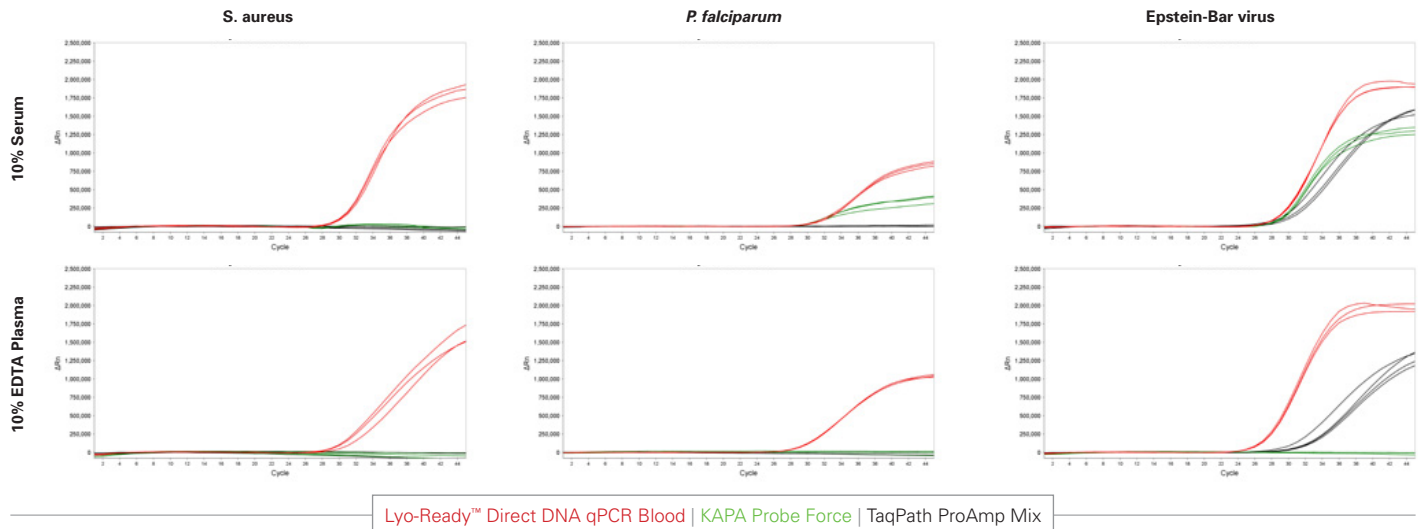
B/ Lyo-Ready™ Direct RNA/DNA qPCR Blood (MDX123)



Anticoagulants and high concentrations of whole blood are known to inhibit qPCR efficiencies. In this experiment, 0% 2%, 5% and 10% whole human blood in the presence of K2-EDTA (orange), sodium heparin (green) or sodium citrate (grey) was tested with (A) Lyo-Ready™ Direct DNA qPCR Blood (MDX122) against Kapa Probe Force (Roche) and TaqPath (Thermo) and (B) Lyo-Ready™ Direct RNA/DNA qPCR Blood (MDX123) against UltraPlex 1-Step ToughMix (QuantaBio) and TaqPath 1-Step Multiplex Master Mix (Thermo). The results demonstrate that the reaction efficiencies of the Lyo-Ready Direct Blood mixes are higher in the presence of inhibitors found in anticoagulants and high concentrations of blood.

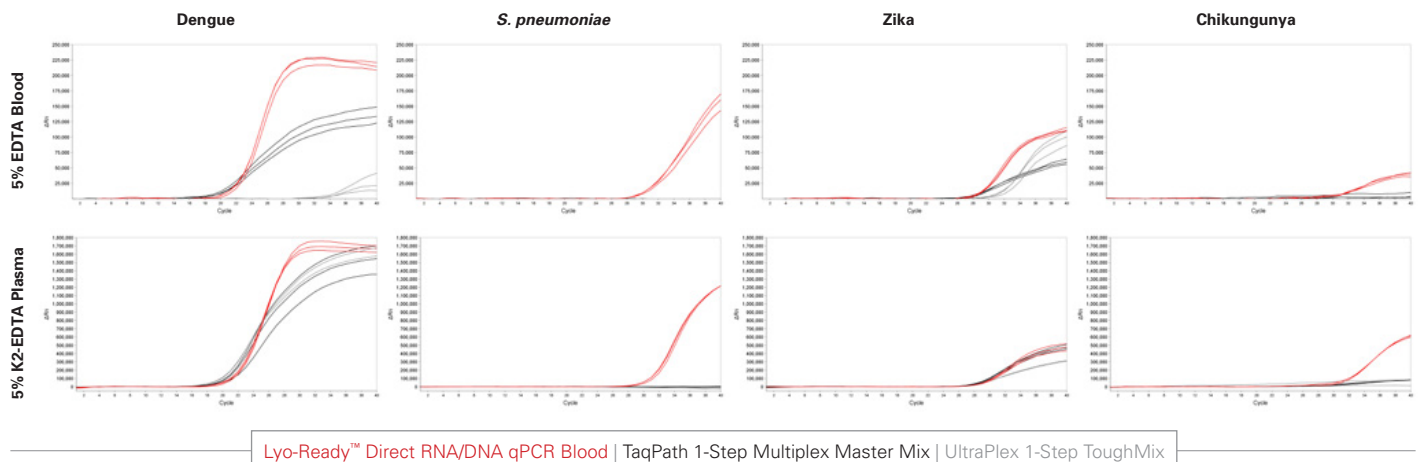
High multiplexing capacity enables the detection of multiple analytes from crude, inhibitor-rich plasma, serum and whole blood samples

Lyo-Ready™ Direct DNA qPCR Blood (MDX122)



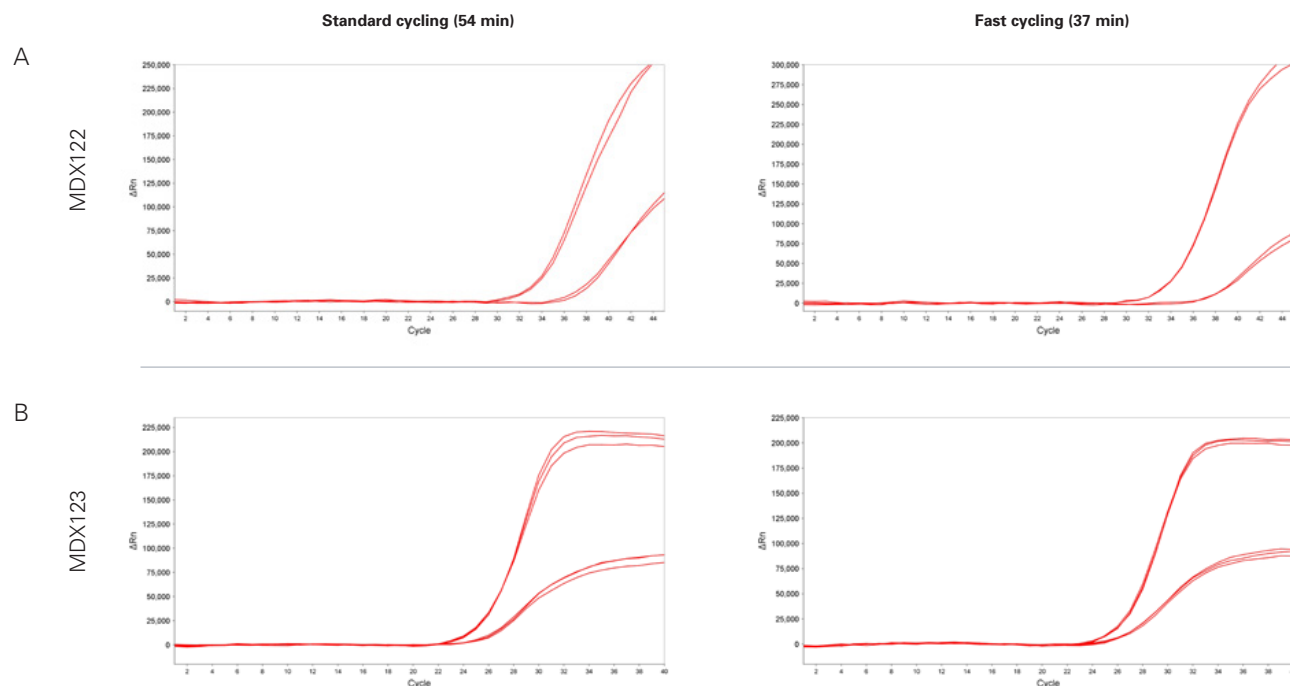
Three diagnostic DNA targets (*Staphylococcus aureus*, *Plasmodium falciparum* and Epstein-Bar virus) were amplified in a triplex reaction using lyophilized Lyo-Ready™ Direct DNA qPCR Blood (MDX122) (red), KAPA Probe Force (green) or TaqPath ProAmp Mix (black) in the presence of 10% EDTA plasma or 10% serum. The results illustrate that Lyo-Ready™ Direct DNA qPCR Blood has higher multiplexing capacity and performance than Kapa Probe Force and TaqPath ProAmp Mix.

Lyo-Ready™ Direct RNA/DNA qPCR Blood (MDX123)



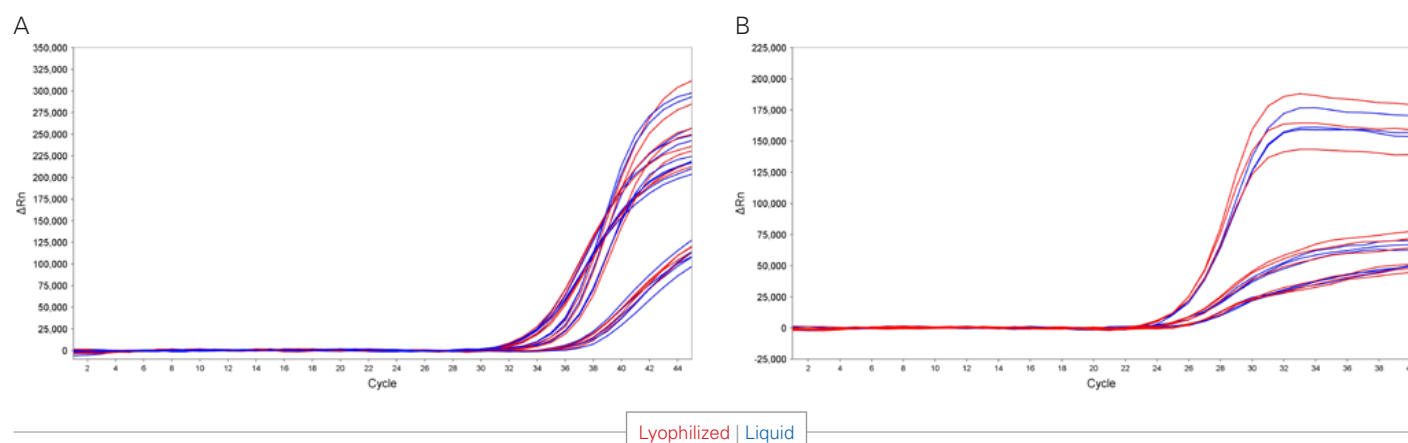
Three viral RNA targets (Dengue, Zika and Chikungunya) and a DNA target (*S. pneumoniae*) were amplified in a quadruplex reaction using lyophilized Lyo-Ready™ Direct RNA/DNA qPCR Blood (red), TaqPath 1-Step Multiplex Master Mix (black) or UltraPlex 1-Step ToughMix (grey) in the presence of 5% EDTA blood or 5% K2-EDTA plasma. The results illustrate that Lyo-Ready™ Direct RNA/DNA qPCR Blood has higher multiplexing capacity and performance than supplier TaqPath or UltraPlex.

Maintains sensitive amplification using fast cycling conditions



The performance of Lyo-Ready™ Direct DNA qPCR Blood (MDX122) on two mammalian DNA targets and Lyo-Ready™ Direct RNA/DNA qPCR Blood (MDX123) on two mammalian RNA targets, in the presence of 5% EDTA blood, were compared using (A) standard qPCR cycling conditions (3 min @ 95°C, 40 cycles of 10 sec @ 95°C and 25 sec @ 60°C), (B) the fastest cycling conditions available on a QuantStudio 7 Flex Real-Time PCR System (3 min @ 95°C, 40 cycles of 1 sec @ 95°C and 1 sec @ 60°C).

Lyophilized format maintains a stable shelf-life for over 18 months



(A) Lyo-Ready™ Direct DNA qPCR Blood (MDX122) and (B) Lyo-Ready™ Direct RNA/DNA qPCR Blood (MDX123) and were lyophilized and the stability of the dried assays (red) was tested after 1 month at 37°C against fresh liquid mix (blue) in triplex assays in the presence of 5% EDTA blood. The lyophilized mixes retained the same performance as the liquid mix. Results suggest that Lyo-Ready Direct Blood mixes have a projected stability of more than 18 months at ambient temperature.

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