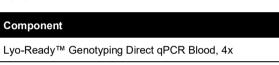


Description

Lyo-Ready[™] Genotyping Direct qPCR Blood is a glycerol-free, one tube formulation compatible with all dual-label probe chemistries for detection of genetic variants, such as single nucleotide variants and copy number variants. The formulation combines the latest advances in buffer chemistry and PCR enhancers, together with an optimized concentration of antibodymediated hot-start polymerase, dNTPs and MgCl₂. Lyo-Ready[™] Genotyping Direct qPCR Blood has been designed for fast, precise, and highly reproducible allelic discrimination and cluster separation with SNP detection assays, even in the presence of PCR inhibitors like blood, serum and plasma. In order to produce room temperature lyophilized qPCR reagents, assay specific primers and probes can be added to Lyo-Ready[™] Genotyping Direct qPCR Blood for subsequent lyophilization.

Kit components





Users Guidelines

The amount of inhibition tolerated by Lyo-Ready™ Genotyping Direct 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 of Lyo-Ready™ Genotyping Direct qPCR Blood and Primer–Probe Mix for lyophilization are given in Table 2. Volumes are indicated per 20 µL final rehydrated reaction.

Table 2

Reagent	Volume
Lyo-Ready™ Genotyping Direct qPCR Blood, 4x	5 μL
Primer-Probe Mix, 20x	1 µL*
Water	xμL
Total volume	Up to 20 µL

*Primer and probe concentration needs to be optimised

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

Lyophilization

For lyophilization protocols, please consult our "Lyophilization & 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 qPCR master mix in the reaction vials with 20 µL 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™ Genotyping Direct qPCR Blood 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	10 min	1	
Denaturation	95 °C	15 s	40-45	
Annealing/Extension*	60-65 °C	30 s	40-43	

*Annealing/Extension temperature needs to be optimised based on probes used.

Associated products

Product	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 RNA/DNA qPCR Blood	MDX123

Technical Support

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