

Lyo-Ready™ Genotyping Direct qPCR FFPE Kit

Product Handling Guide

Shipping:	On Dry/Blue Ice
Catalog number:	MDX168
Batch No.:	See vial
Concentration:	4x

Store at -20 °C



Storage and stability:

Lyo-Ready™ Genotyping Direct qPCR FFPE Kit is shipped on dry/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™ Genotyping Direct qPCR FFPE Kit 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 and further manufacturing use only.

Description

Lyo-Ready™ Genotyping Direct qPCR FFPE 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 antibody-mediated hot-start polymerase, dNTPs and MgCl₂. Lyo-Ready™ Genotyping Direct qPCR FFPE has been designed for fast, precise, and highly reproducible allelic discrimination and cluster separation with SNP detection assays, even in the presence of crude FFPE tissue extracts. Lyo-Ready™ Genotyping Direct qPCR FFPE can detect DNA from FFPE tissue digested in a single tube, without the need for column purification of DNA, greatly reducing the risk of sample loss and contamination. In order to produce room temperature lyophilized qPCR reagents, assay specific primers and probes can be added to Lyo-Ready™ Genotyping Direct qPCR FFPE for subsequent lyophilization.

Kit components

Table 1

Component
Lyo-Ready™ Genotyping Direct qPCR FFPE, 4x
Buffer A
Buffer B

Users Guidelines

FFPE tissue extract preparation

- Cut a piece of FFPE tissue and remove paraffin using a scalpel. Weigh 3.5 mg of tissue and transfer it into a clean 1.5 mL microfuge tube.
- Add 1 mL of Xylene (not supplied) to the sample, vortex for 10 sec and then centrifuge at full speed for 2 minutes at room temperature.
- Carefully remove supernatant by pipetting.
- Add 1 mL ethanol (96-100%) to the pellet, vortex for 10 sec and then centrifuge at full speed for 2 minutes at room temperature.
- Carefully remove supernatant by pipetting.
- Air-dry the tube for 10 minutes at room temperature.
- Add 20 µL Extraction Buffer A, 10 µL Extraction Buffer B and 70 µL of water and mix well.
- Incubate for 60 minutes at 75°C. (Regularly vortex the sample vigorously, at least four times during the incubation period).
- Heat for 60 minutes at 90°C.
- Cool down samples on ice and then centrifuge at full speed for 5 minutes
- Carefully transfer supernatant into a clean tube.
- Dilute the digested sample with water according to requirements of the downstream application.

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 FFPE, 4x	5 µL
Primer-Probe Mix, 20x	1 µL*
Water	x µL
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	

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

Associated products

Product	Cat. No.
Tissue Extract-PCR Buffers	MDX004
Lyo-Ready™ Genotyping Direct qPCR Blood	MDX128

Technical Support

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