Genotyping Blood qPCR Mix

Product Handling Guide

Shipping:	Blue Ice
Catalog number:	ODX001
Batch No.:	See vial
Concentration:	4x

Store at –20 °C

Storage and stability:
Genotyping Blood qPCR Mix 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. Genotyping Blood qPCR Mix and its components are extensively tested for activity, efficiency, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination.

Notes:
For research and further manufacturing use only.

Description

Genotyping Blood qPCR Mix is 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 $MgCl_2$. Genotyping Blood qPCR Mix 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.

Kit components

Table 1

Component
Genotyping Blood qPCR Mix, 4x

General considerations

To help prevent any carry-over DNA contamination, we recommend that separate areas are maintained for reaction set-up, PCR amplification and any post-PCR gel analysis. It is essential that any tubes containing amplified PCR product are not opened in the PCR set-up area.

Primer and Probes: The following information relates to the design and setup of TaqMan probe-based PCR. When using other probe types, please refer to the appropriate literature.

The sequence, concentration ratio of the primers to probes and the amplicon length, can be critical for specific amplification, yield and overall efficiency of any qPCR. We strongly recommend taking the following points into consideration when designing and running your qPCR:

- Primers should have a melting temperature (Tm) of approximately 60° C; the Tm of the probe should be approximately 10°C higher than that of the primers.
- Optimal amplicon length should be 80-200 bp
- Final primer concentration of 900 nM is suitable for most reactions, however to determine optimal concentration we suggest titrating in the range of 0.2-1 µM.
- Final concentrations of 200 nM for each probe are adequate for most reactions; we recommend the final probe concentrations are at least 2 -fold lower than the primer concentration.
- We recommend the use of an equimolar concentration for the primers. The ratio of the probes should be optimized , but the probes ratio should be optimized.

PCR controls: Always include a no-template control, by omitting template from the reaction. To ensure reliable genotype calling, positive controls containing genomic DNA samples of known genotype should be run with each assay.

Procedure

The final volumes given below are based on a standard 20 µL final reaction mix and can be scaled accordingly.

Reagent	Volume	Final Concentration
Genotyping Blood qPCR Mix, 4x	5 µL	1x
18 µM Forward Primer	1 µL	0.9 µM
18 µM Reverse Primer	1 µL	0.9 µM
4 µM Allele 1 Probe*	1 µL	0.2 µM
4 µM Allele 2 Probe*	1 µL	0.2 µM
Template	x µL**	
Total Volume	**Up to 20 µL**	

**The ratio between the two probes should be optimized.*
*** Up to 10% whole blood or 20% serum or plasma can be used, however the amount of inhibition tolerated by Genotyping Blood qPCR Mix is variable depending on several factors, including assay design and sample quality. For this reason, an initial sample titration is recommended.*

Suggested qPCR conditions: The following qPCR conditions are suitable for the Genotyping Blood qPCR Mix with amplicons of up to 200 bp. However, the cycling conditions can be varied to suit different machine-specific protocols. It is not recommended to use annealing temperatures below 60°C.

Step	Temperature	Time	Cycles
Polymerase activation	95 °C	10 min	1
Denaturation	95 °C	15 s	45
Annealing/Extension**	60-65 °C	30 s	

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

Troubleshooting guide

Problem	Possible Cause	Recommendation
No or low amplification trace resulting in single indistinguishable cluster in scatter plot	Error in protocol setup	Verify that correct reagent concentrations, volumes, dilutions and storage conditions have been used
	Suboptimal primer design	Use primer design software or validated primers. Test primers on a control template. Ensure no SNP is present in the primer region.
	Incorrect concentration of primers	Use a final primer concentration that is at least twice that of the probe.
	Template degraded	If necessary, re-isolate your template from sample material or use freshly prepared dilutions.
	Primers degraded	Use newly synthesized primers.
	Template contaminated with PCR inhibitors	Further dilute template before PCR or re-purify template and resuspend it in PCR-grade H_2O.
	Template concentration too low	Increase concentration used.
	Cycling conditions not optimal	Increase extension/annealing times, increase cycle number.
No clusters in scatter plot	Template contaminated with PCR inhibitors	Further dilute template before PCR or purify template and resuspend it in PCR-grade H_2O.
	Template degraded	If necessary, re-isolate your template from sample material or use freshly prepared dilutions.
	Wrong reporter dye selected	Check dye settings.
More than 3 clusters in scatter plot	More than one SNP	Check the SNP database (http://www.ncbi.nlm.nih.gov/snp) for the presence of additional or newly discovered SNPs.
	More than one copy of SNP or SNP is multi-allelic	Sequence the DNA samples and compare.
	Sample contamination	Test the sample integrity by running the samples on alternative assays.
Clusters in scatter plot are close together	Too many cycles run	Re-run the PCR with fewer cycles.
	Template degradation	If necessary, re-isolate your template from sample material or use freshly prepared dilutions.
	Probe degradation	Use new reagents and re-run the PCR.
Clusters appear "stretched" in scatter plot	Variable sample	Run samples on agarose gel to check for degradation. Re-check DNA concentrations and ensure comparable amounts are used for each sample.
	PCR inhibitors	Dilute the DNA sample.
	Poor reagent delivery or evaporation during run	Visually inspect and check for volume variation in each well. Repeat the assay for any sample with the wrong volume. Before each run, check the reaction plate is sealed properly. Refer to instrument guidelines to see if you need to use a compression pad.
	Air bubbles in reaction mix	Centrifuge reaction samples/plate prior to running on a real-time instrument.
	Poor mixing	Mix reactions and re-run the PCR.

Associated Products

Product	Cat. No.
Liquid Biopsy Blood DNA qPCR Mix	ODX002
Liquid Biopsy Blood RNA/DNA qPCR Mix	ODX003
Liquid Biopsy Urine DNA qPCR Mix	ODX004
Liquid Biopsy Stool DNA qPCR Mix	ODX005

Technical Support

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

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