Lyo-Ready™ Genotyping Direct qPCR Blood

Blood inhibitor tolerant master mix enabling exceptional cluster resolution with clear allele discrimination

Lyo-Ready™ Genotyping Direct qPCR Blood is a glycerol-free, one-tube formulation optimized for SNP genotyping applications with dual-label probes.

Genotyping is the process of determining genetic variations among individuals in a population. SNPs (single nucleotide polymorphisms) or point mutations are the most common types of genetic variation and comprise the major part of the phenotype diversity between individuals. Most SNPs have no effect on health or development. However, they can be used in to predict an individual's response to certain drugs (pharmacogenetics), susceptibility to environmental factors such as toxins, and risk of developing diseases (diagnostics). SNPs can also be used to track the inheritance of disease-associated genetic variants within families.

SNPs can be responsible for both resistance or susceptibility to a certain disease and once individual SNPs have been identified by sequencing, they can be used for phamacogenetics, in evaluating and predicting a patient's response to treatment and risk of adverse events, or for diagnostics, such as in cystic fibrosis screening.

Clinical screening of SNPs requires large-scale multiplexed genotyping, which traditionally relies on DNA extraction from complex samples such as blood, to achieve sensitive DNA amplification. However, blood contains several PCR inhibitors such as immunoglobulin G, hemoglobin, lactoferrin and leukocyte DNA that can inhibit the reaction or reduce a reaction's efficiency. Meridian's Lyo-Ready™ Genotyping Direct qPCR Blood is a 4x inhibitor-resistant mix designed for highly specific and sensitive amplification for genotyping sequence variants, even in the presence of PCR inhibitors found in blood, serum or plasma.

The combination of two key features, inhibitor-tolerance and lyophilization compatibility, make Lyo-Ready™ Genotyping Direct qPCR Blood ideal for high-throughput and point-of-care genotyping assays. The optimized mix achieves exceptional cluster resolution with clear allele discrimination, even with challenging SNP targets.

Lyo-Ready™ Genotyping Direct qPCR Blood has been optimized to provide highly specific allelic discrimination as demonstrated by excellent cluster separation, even in the presence of PCR inhibitors found in blood, serum or plasma. Furthermore, it can be used in a liquid or lyophilized format to create ambient-temperature stable assays, making it ideal for point-of-care (POC) devices.

PRODUCT	CAT NO.	VOLUME	REACTIONS
Lyo-Ready™ Genotyping Direct qPCR Blood, 4x	MDX128	5 mL	1,000 Rxns
		50 mL	10,000 Rxns

Product Highlights

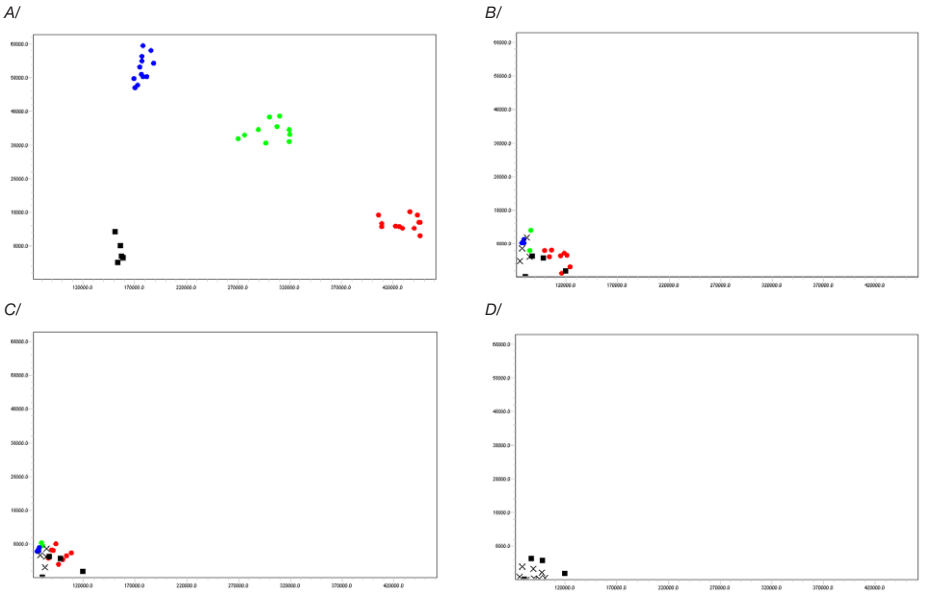
- Ultra-sensitive detection down to less than 1 copy using direct amplification protocols
- Inhibitor-tolerant to PCR inhibitors found in whole blood, serum, plasma and anticoagulants
- Tight fluorescence clusters with clear allele discrimination, perfect for difficult SNPs
- Mixes can be used as a liquid or lyophilized to extend assay shelf-life, ideal for high-throughput or point of care POC testing
- Compatible with a range of lyophilization protocols

Performance Data

Tighter fluorescence clusters with clearer allele discrimination compared to other commercially available mixes with samples containing a range of inhibitors found in blood

A) K2-EDTA Whole Human Blood

SNP differences between two strains of Epstein-Barr Virus (EBV) were tested using Lyo-Ready™ Genotyping Direct qPCR Blood Mix, Roche Kapa Probe Force, ThermoFisher TaqPath™ and Qiagen Type-it Fast SNP Probe PCR Kits.



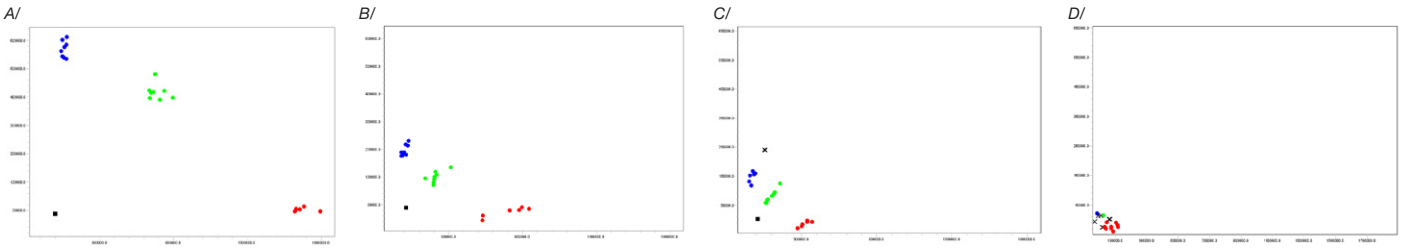
10% K2-EDTA whole human blood was tested with A/ Lyo-Ready™ Genotyping Direct qPCR Blood Mix, B/ Roche Kapa Probe Force, C/ ThermoFisher TaqPath™ and D/ Qiagen Type-it Fast SNP Probe PCR Kits, using EBV targets. Homozygous samples for allele A (red) and allele C (blue) and heterozygous samples for allele A/C (green) were compared with a NTC (**black**) and x for undetermined. The results illustrate ability of Lyo-Ready™ Genotyping Direct qPCR Blood to form tight clustering and so accurate allelic discrimination in the presence of whole blood unlike the other mixes.

Allele A | Allele C | Allele A/C | NTC

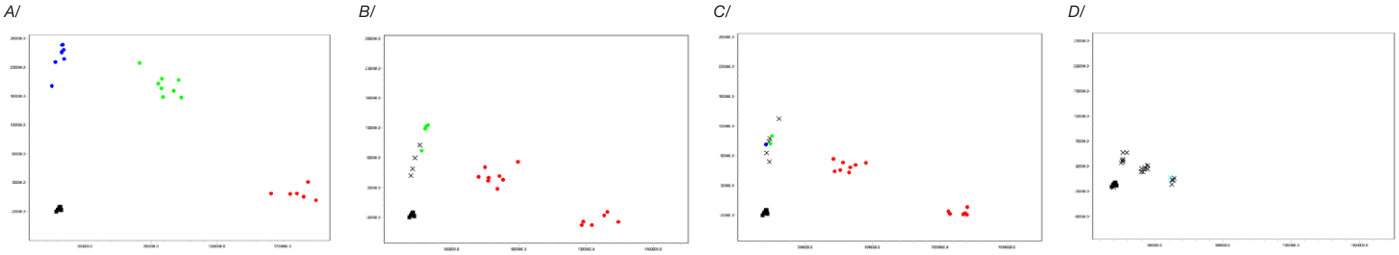
B) Plasma and Serum

The ability to detect two autosomal recessive variants, Rs67376798 a 2846A›T variant and Rs3918290 a C›T variant, in 20% plasma or 20% serum were compared using Lyo-Ready™ Genotyping Direct qPCR Blood, Kapa Probe Force, TaqPath™ and Type-it Fast Kits.

20% human plasma



20% human serum

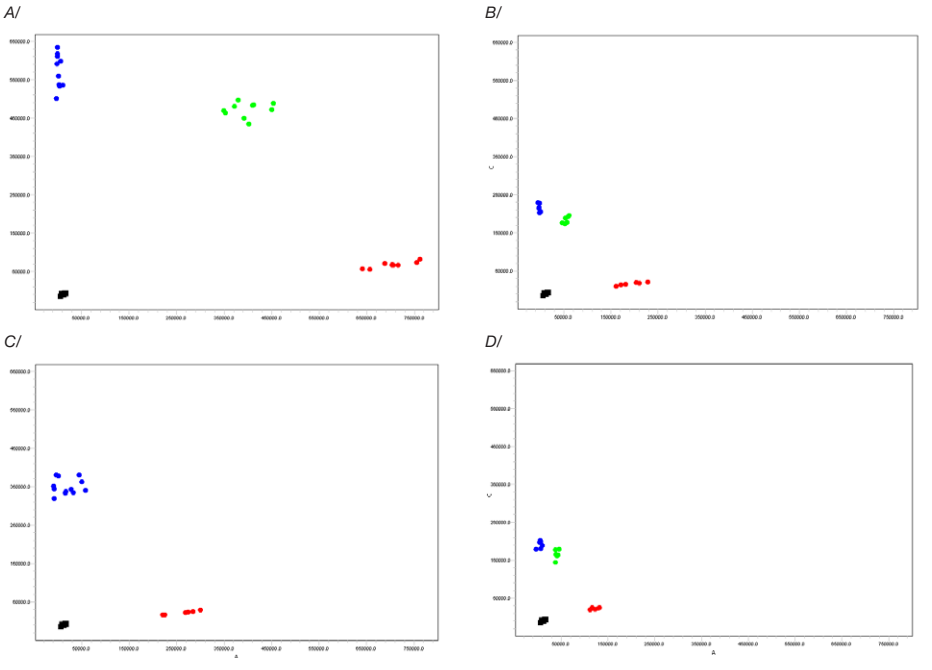


Allele A | Allele C | Allele A/C | NTC

20% human plasma was tested using rs67376798 drug metabolism target and 20% human serum was tested using rs3918290 drug metabolism target, with A/ Lyo-Ready™ Genotyping Direct qPCR Blood, B/ Kapa Probe Force, C/ TaqPath™ and D/ Type-it Fast Kits. Homozygous allele A (red) and allele C (blue) and heterozygous allele A/C (green) with a NTC (**black**) and x for undetermined. Again, the results illustrate ability of Lyo-Ready™ Genotyping Direct qPCR Blood to form tighter, more distinct clustering and so more accurate allelic discrimination in the presence of plasma and serum.

C) Hemin

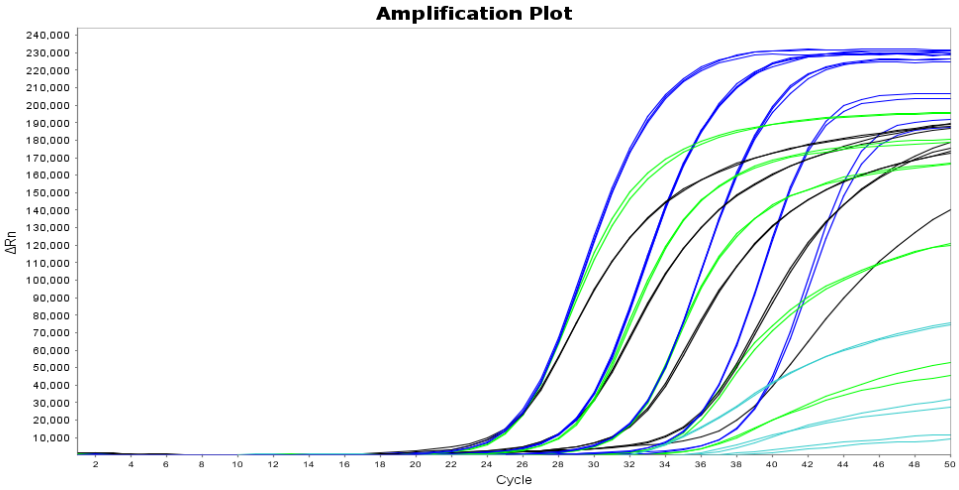
Amplification of CYP3A4, a steriod hydroxalyase, was compared in the presence of 2 mM Hemin using Lyo-Ready™ Genotyping Direct qPCR Blood, Kapa Probe Force, TaqPath™ and Type-it Fast Kits.



Allele A | Allele C | Allele A/C | NTC

2 mM Hemin was tested with A/ Lyo-Ready™ Genotyping Direct qPCR Blood, B/ Kapa Probe Force, C/ TaqPath™ and D/ Type-it Fast Kits using CYP3A4, a steroid hydroxylase. Homozygous allele A (red) and allele C (blue) and heterozygous allele A/C (green) with a NTC (**black**) and x for undetermined. Genotyping analysis from the Lyo-Ready™ Genotyping Direct qPCR Blood Mix illustrate accurate allelic discrimination clustering in the presence of Hemin.

Superior Sensitivity and Reproducibility in Samples Containing 10,000 copies to 1 copy of DNA in 10% Plasma



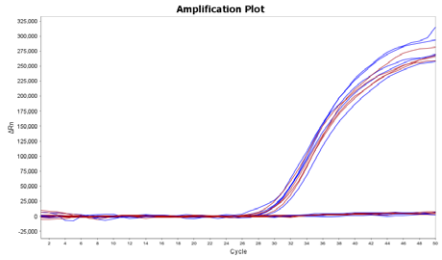
MDX128 | KAPA Probe Force | TaqPath™ | Type-it Fast

Activity of Lyo-Ready™ Genotyping Direct qPCR Blood (blue) was compared to KAPA Probe Force (green), TaqPath™ (**black**), and Type-it Fast (light blue) kits in a qPCR assay, using a 10-fold serial dilution of VLP DNA (10,000, 1000, 100, 10 and 1 copies respectively), in the presence of 10% plasma. The results illustrate that Lyo-Ready™ Genotyping Direct qPCR Blood has significantly higher sensitivity and reproducibility in assays detecting high or low copies of DNA template and in the presence of PCR inhibitors, resulting in tighter, more discrete, well-defined cluster.

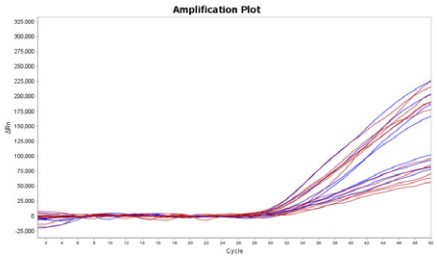
Ability to Extend Shelf-life to Over 12 Months in Lyophilized Format

A) Amplification plot

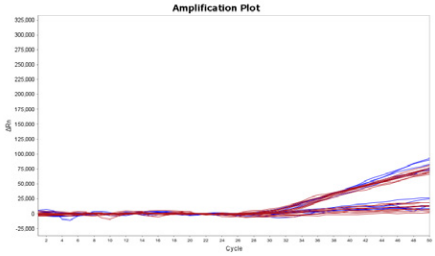
Homozygous A/A



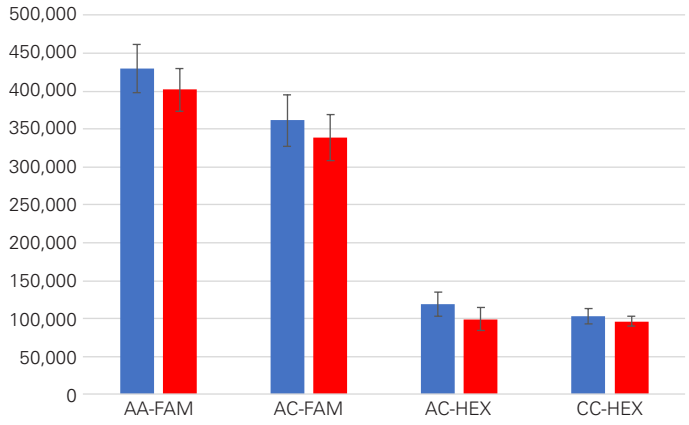
Heterozygous A/C



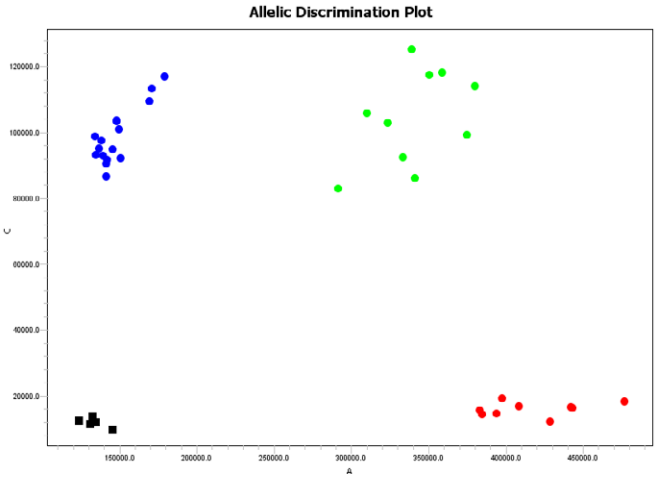
Homozygous C/C



B) End Fluorescence



C) Allelic discrimination



Liquid | Lyophilized

Lyo-Ready™ Genotyping Direct qPCR Blood was lyophilized, and the stability of the dried assays (red) was tested after 1 month at 37°C against fresh liquid mix (blue) in the presence of 5% EDTA Blood (A/ and B/). The lyophilized mixes retained the same performance as the liquid mix (C/ homozygous allele A (red) and allele C (blue) and heterozygous allele A/C (green) with a NTC (black) and x for undetermined). Results suggest that Lyo-Ready™ Genotyping Direct qPCR Blood mix has a projected stability of more than 18 months at ambient temperature

Ordering information:

USA
5171 Wilfong Road
Memphis, Tennessee 38134
Phone: +1 901-382-8716
Fax: +1 901-333-8223

Email: info@meridianlifescience.com
Orders: orders@meridianlifescience.com
www.meridianbioscience.com/lifescience

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