Lyo-Ready™ Genotyping Direct qPCR FFPE

FFPE sample compatible master mix enabling exceptional cluster resolution with clear allele discrimination.

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 variations.

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. These SNPs can be responsible for both resistance or susceptibility to a certain disease. Consequently, 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.

Although blood is an ideal source for genotyping analysis as it is one of the most commonly used specimens used for laboratory diagnostic testing, formalin-fixed paraffin-embedded (FFPE) tumor tissues are also widely used and constitute valuable resources for retrospective studies due to the possibility of long-term storage at room temperature. The DNA yield and quality from such sources, however, may be poor due to cross-linking of the DNA and its fragmentation. Despite the extremely time-consuming and labour-intensive workflows, success rate of genotyping assays using DNA purified from such samples remains low.

To minimize the workflow to isolate DNA from FFPE tissue, Meridian has created the Lyo-Ready™ Genotyping Direct qPCR FFPE, this allows digestion of the tissue in a single tube, without the need for purification, greatly reducing the risk of sample damage, loss and contamination whilst simplifying and accelerating the analysis process. Furthermore, it can be used in a liquid format or lyophilized format to create ambient-temperature stable assays, making it ideal for high throughput.

PRODUCT	CAT NO.	VOLUME	REACTIONS
Lyo-Ready™ Genotyping Direct qPCR FFPE, 4x	MDX168	5 mL	1,000 Rxns
		50 mL	10,000 Rxns

Product Highlights

- Ultra-sensitive detection down to 1 copy using direct amplification protocols
- Inhibitor-tolerant to PCR inhibitors found in FFPE tissue
- Tight fluorescence clusters with clear allele discrimination, perfect for difficult SNPs identification
- Mixes can be used in liquid or lyophilized formats to extend assay shelf-life
- Compatible with a range of lyophilization protocols

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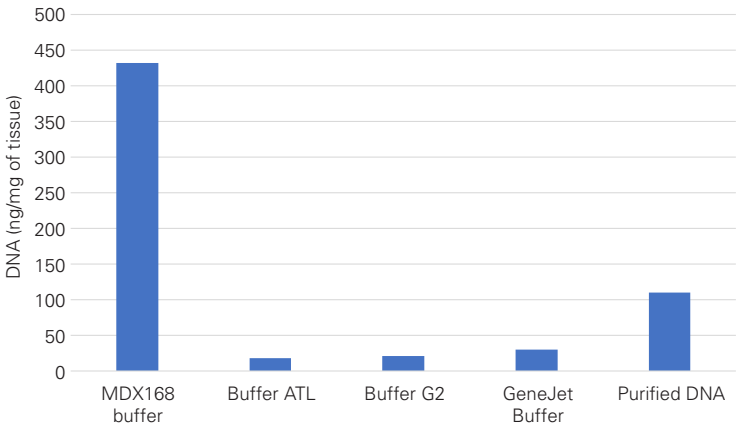
Performance Data

Lyo-Ready™ Genotyping Direct qPCR FFPE combines the latest advances in buffer chemistry and PCR enhancers, together with an optimized antibody-mediated hot-start polymerase, dNTPs and $MgCl_2$, along with a two-tube tissue extraction buffer, to release the DNA, for fast, precise, and highly reproducible allelic discrimination and cluster separation with SNP detection assays, even in the presence of PCR inhibitors presence in crude FFPE tissue extracts.

Higher DNA Extraction Yield with Meridian's MDX168 Extraction Buffer



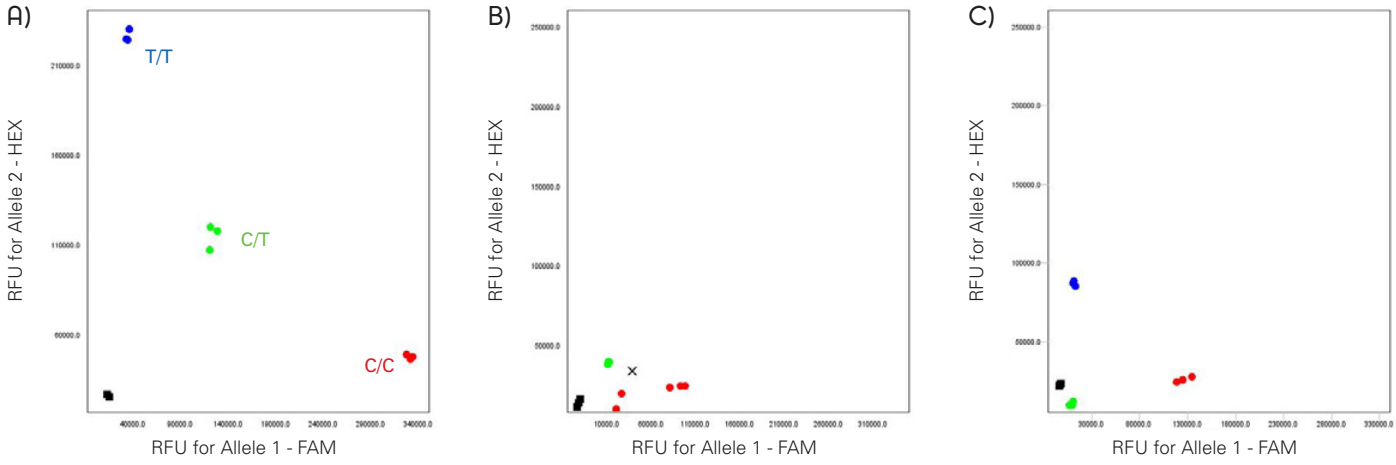
7 mg of FFPE lung tissue digested using MDX168 Extraction Buffer, Buffer ATL (Qiagen), Buffer G2 (Qiagen) and GeneJet FFPE Kit Extraction Buffer (ThermoFisher) and compared to purified DNA obtained using the Qiagen QIAamp FFPE Tissue DNA extraction kit (as a reference). The results show that a significantly higher quantity of DNA was obtained from tissue digested with MDX168 Extraction Buffer compared to purified DNA obtained with an extraction/ purification kit or tissues digested with other commercially available buffers. These results highlight the superior DNA extraction yield obtained from FFPE tissue with MDX168 Extraction Buffer. The DNA stored in the MDX168 Extraction Buffer is stable up to 24 hours at room temperature and 3 days at 4 °C.

Tighter fluorescence clusters with clearer allele discrimination compared to other commercially available mixes with FFPE samples

KIT24 and PDGFRA (Platelet Derived Growth Factor Receptor Alpha) are both part of a receptor tyrosine kinases (RTKs) signalling pathway. Ataxia-telangietasia mutated (ATM) is a kinase that acts upstream of p53 and controls a DDR pathway critical to resolving double-stranded DNA breaks. Cancer-associated SNP mutations can be found in lung tumour tissue for all three of these genes.

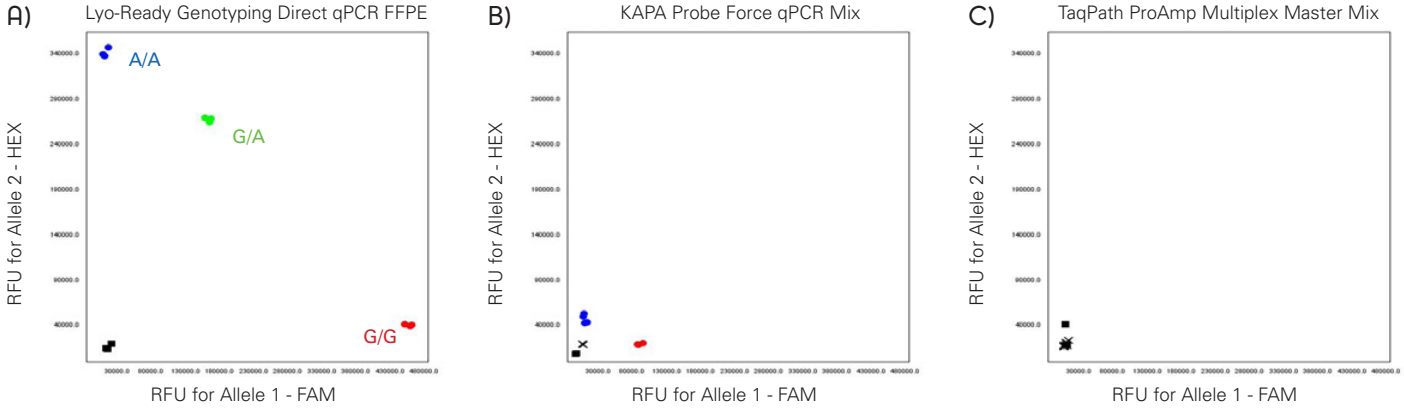
In the graphs below, A/ Lyo-Ready Genotyping Direct qPCR FFPE (MDX168), B/ KAPA Probe Force qPCR Mix (Roche) and C/ TaqPath™ ProAmp Multiplex Master Mix (ThermoFisher). The results illustrate the ability of Lyo-Ready Genotyping Direct qPCR FFPE to form tighter, more distinct clustering and therefore more accurate allelic discrimination in the presence of crudely extracted FFPE lung tissue than other genotyping kits.

KIT24_48 gene mutations



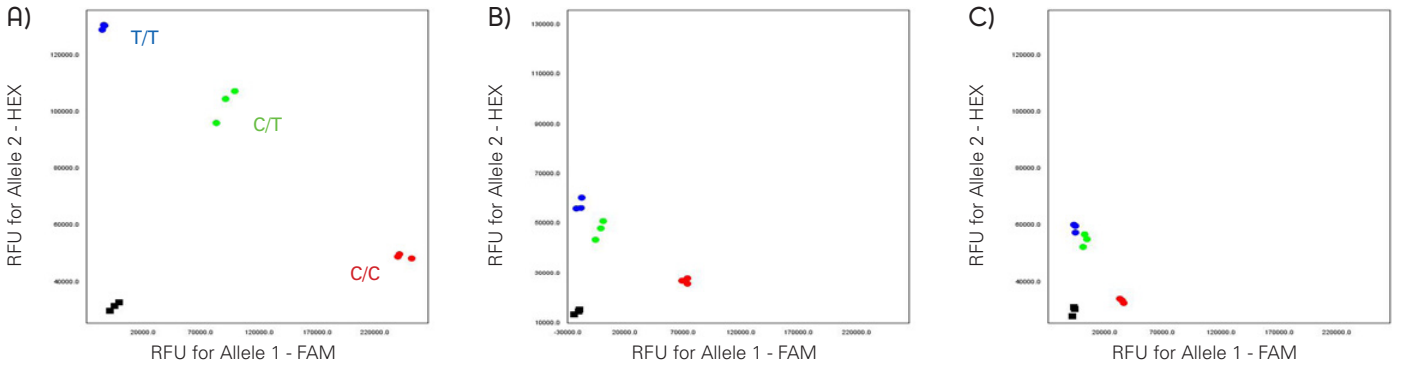
Healthy FFPE lung tissue (WT) and lung tumour tissue containing the cancer associated KIT24_48 mutation (Mutant) were digested using MDX168 extraction buffers. 5.2 ng of DNA from digested tissues were in the reaction. Homozygous Allele C (red) and allele T (blue) and heterozygous Allele C/T (green) with a NTC (**black**) and x for undetermined.

PDGFRA1 gene mutations



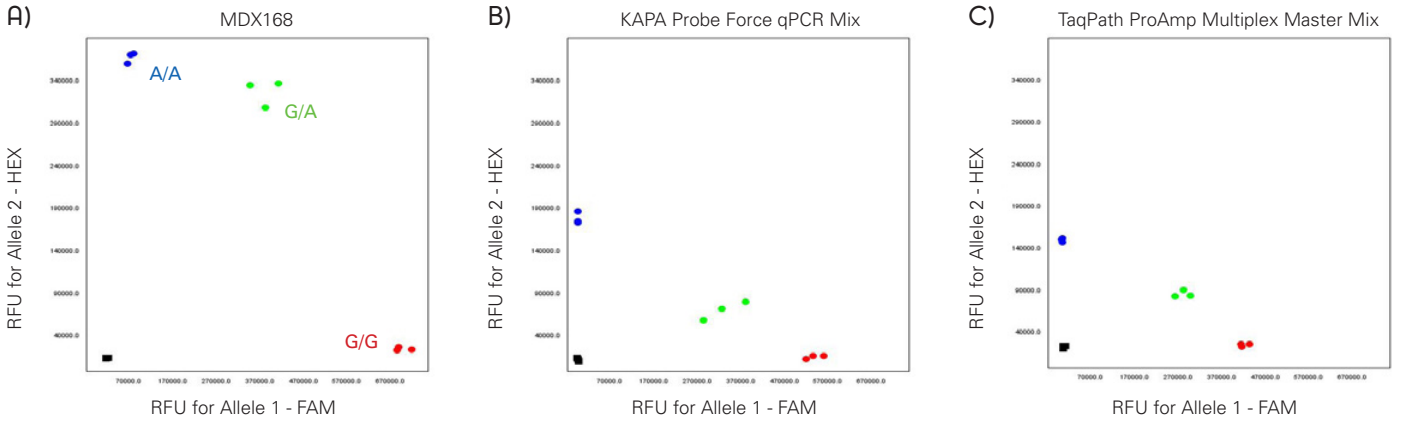
Healthy FFPE lung tissue (WT) and lung tumour tissue containing the tumor angiogenesis associated PDGFRA1 mutation (Mutant) were digested using MDX168 extraction buffers. 5.2 ng of DNA from digested tissues were in the reaction. Homozygous Allele G (red) and allele A (blue) and heterozygous Allele G/A (green) with a NTC (black) and x for undetermined.

ATM gene mutations



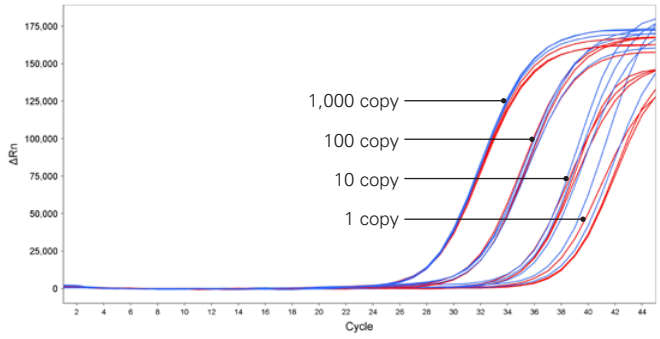
Healthy FFPE lung tissue (WT) and lung tumour tissue containing the cancer associated ATM17 mutation (Mutant) were digested using MDX168 extraction buffers. 1.3 ng of DNA from digested tissues were in the reaction. Homozygous Allele C (red) and allele T (blue) and heterozygous Allele C/T (green) with a NTC (black) and x for undetermined.

Crudely extracted FFPE DNA vs Purified FFPE DNA - PDGFRA1 Assay



1.3 ng of DNA from lung tumour FFPE tissue containing the tumoral angiogenesis associated PDGFRA1 mutation (Mutant) was either crudely extracted using the MDX168 Extraction Buffer and analysed with A/ Lyo-Ready Genotyping Direct qPCR FFPE (MDX168) or purified using Qiagen QIAamp DNA FFPE Tissue Extraction Kit and analysed with B/ KAPA Probe Force qPCR Mix (Roche) and C/ TaqPath ProAmp Multiplex Master Mix (ThermoFisher). Homozygous Allele G (red) and allele A (blue) and heterozygous Allele G/A (green) with a NTC (black) and x for undetermined. The results illustrate that Lyo-Ready Genotyping Direct qPCR FFPE (MDX168) shows greater allelic discrimination and tighter clustering even in the presence of crude FFPE tissue extracts in comparison to other commercially available master mixes using purified gDNA.

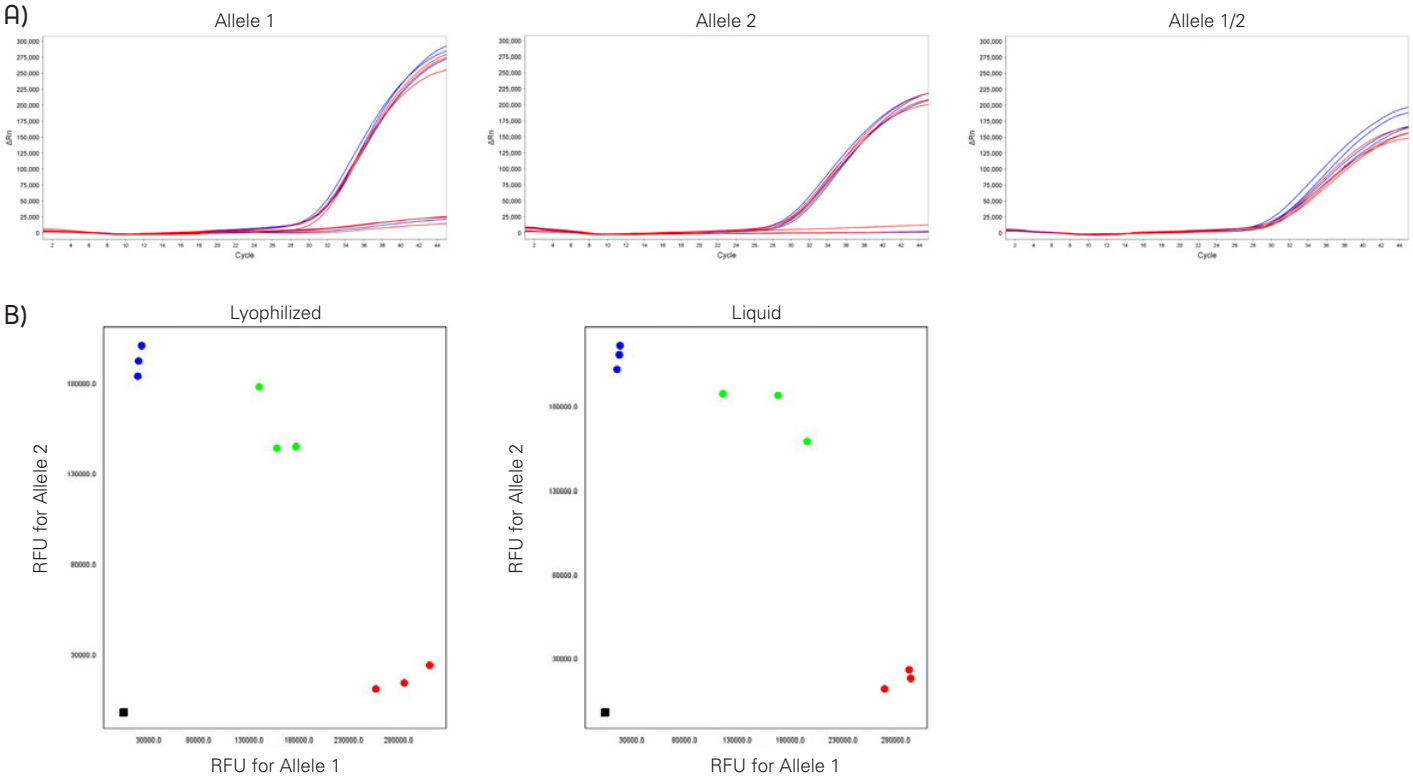
Detect down to 1 copy DNA in presence of inhibitors



Activity of Lyo-Ready™ Genotyping Direct qPCR FFPE in liquid format (blue) and lyophilized (red) in a qPCR assay, using a 10-fold serial dilution of synthetic DNA (1,000, 100, 10 and 1 copies respectively), in the presence of 20% MDX168 Extraction Buffer. The results illustrate that the lyophilized Lyo-Ready™ Genotyping Direct qPCR FFPE retains the ability to efficiently amplify to the same level as the liquid mix with the same level of sensitivity and reproducibility.

Liquid | Lyophilized

Lyophilized mixes maintain a stable shelf life for up to 18 months



Lyo-Ready™ Genotyping Direct qPCR FFPE was lyophilized, and its stability tested in an accelerated stability study. The lyophilized mix (red) was incubated at 37°C for 1 month and tested against the fresh liquid mix (blue) in a standard dual-labelled probe genotyping assay in the presence of 20% MDX168 Extraction Buffer. Results show that the lyophilized mix retain A) the Ct-values and end-fluorescence in real-time PCR and B) allelic discrimination as the liquid mix following accelerated stability tests with projected 18-months stability at ambient temperature.

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