Lyo-Ready™ Genotyping Direct DNA qPCR Stool

Enabling sensitive detection of SNPs from stool samples

Lyo-Ready™ Genotyping Direct qPCR Stool is a glycerol-free, one-tube formulation, compatible with all dual-label probe chemistries for the detection of genetic variants, such as single nucleotide variants (SNPs) and copy number variants. It is ideal for a number of applications including infectious disease testing, cancer biomarker detection, microbiome analysis, antibiotic resistance, and pharmacogenomics.

Genotyping assays are used to diagnose genetic disorders, identify disease risk factors, and guide treatment decisions. Point mutations, including single nucleotide polymorphisms (SNPs), are the most common type of genetic variation in the human genome. They play a significant role in generating genetic diversity among individuals, and in increasing or decreasing an individual's risk of developing diseases such as cancer. These point mutations also significantly impact organisms found within the gut (microbiome) exacerbating the severity of diseases and changing the way they need to be treated.

Genotyping from stool samples can be challenging because stool material can contain a lower concentration of DNA compared to other biological samples such as blood or saliva. In addition, the presence of inhibitors and the sensitivity of the genotyping method used can influence the success of a genotyping assay. Advances in genotyping technologies have improved the performance of stool assays, and opened up avenues for stool testing in several disease areas including:

1. **Infectious Disease:** Gastrointestinal infections such as shiga-like toxin producing E. coli or antibiotic resistance in *H. pylori*
2. **Cancer Biomarker Detection:** Early-stage screening for cancers such as bowel cancer
3. **Pharmacogenomics:** Identification of genetic variations that influence an individual's response to certain drugs
4. **Research & Epidemiology Studies:** Population genetics research to provide insights into the genetic basis of various traits or diseases, particularly in wildlife and livestock

Meridian's new Lyo-Ready Genotyping Direct qPCR Stool is a mix that is designed for fast, precise, and reproducible allelic discrimination and cluster separation. Its advanced formulation enables highly sensitive amplification of SNPs and other point mutations from stool samples and overcomes inhibitors such as bile salts that can damage DNA and inhibit PCR reactions. Furthermore, it can be used in a wet format or lyophilized to create ambient-temperature stable assays, making it ideal for point-of-care (POC) devices.

Product Highlights

- Ultra-sensitive detection down to single copies using direct amplification protocols
- Inhibitor-tolerant to PCR inhibitors found in stool (e.g. bile salts) and in transport media (such as Cary-Blair medium)
- Forms 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 point-of-care testing)
- Compatible with a range of lyophilization protocols

PRODUCT	CAT NO.	VOLUME	REACTIONS
Lyo-Ready™ Genotyping Direct DNA qPCR Stool, 4x	MDX148	5 mL	1,000 Rxns
		50 mL	10,000 Rxns

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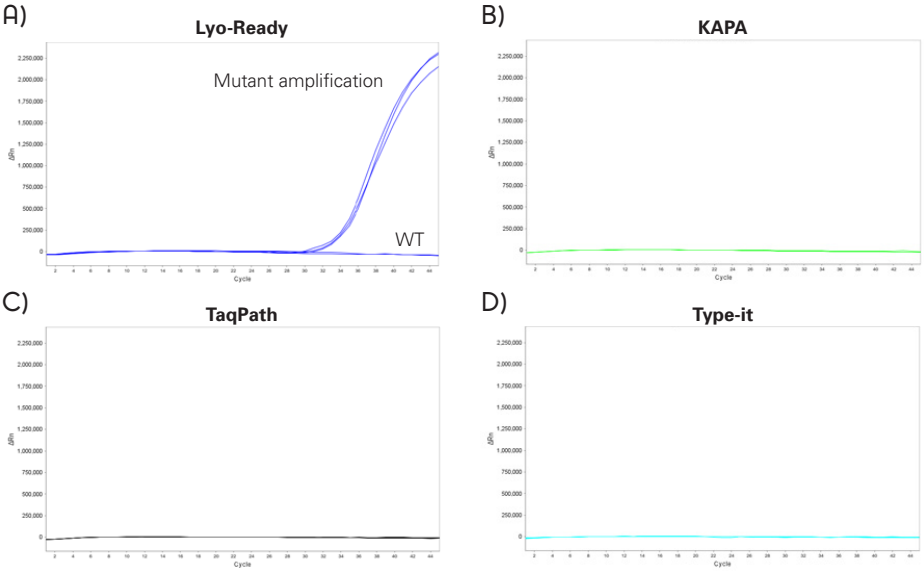
Performance Data

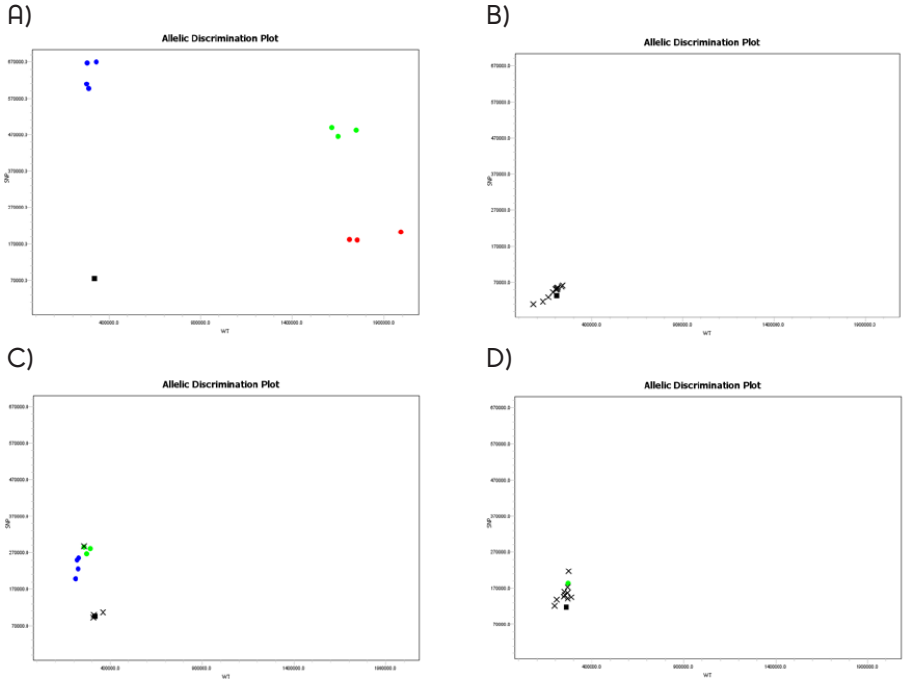
Lyo-Ready™ Genotyping Direct qPCR Stool combines the latest advances in buffer chemistry and PCR enhancers, together with an optimized antibody-mediated hot-start polymerase, for fast, precise, and highly reproducible allelic discrimination and cluster separation with SNP detection assays, even in the presence of PCR inhibitors found in stool.

Detection of Colorectal Cancer-Associated BRAF V600E Mutation from 15 mg/mL Human Stool



Detection of colorectal cancer-associated BRAF V600E mutation (SNP accession number: rs113488022) using A) Lyo-Ready™ Genotyping Direct qPCR Stool (MDX148), B) KAPA Probe Force qPCR Mix (Roche), C) TaqPath™ ProAmp™ Multiplex Master Mix (ThermoFisher) and D) Type-it Fast SNP Probe PCR Kits (Qiagen) in presence of 15 mg/mL human stool. BRAF V600E (val600-to-glu) mutation is a prognostic and predictive biomarker of aggressive tumor growth and treatment with EGFR inhibitors. BRAF testing is recommended for stage IV, metastatic colorectal cancer (mCRC) before initiating therapy with anti-EGFR inhibitors, or when chemotherapy treatment stops working[1]. The results illustrate the sensitivity of Lyo-Ready™ Genotyping Direct qPCR Stool to detect the presence of low levels of clinically relevant mutations directly from stool samples.

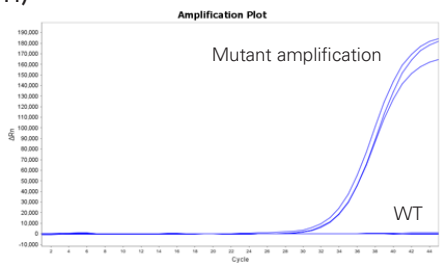
Detection of Gastric Cancer-Associated p53 Mutation from 20 mg/mL Human Stool



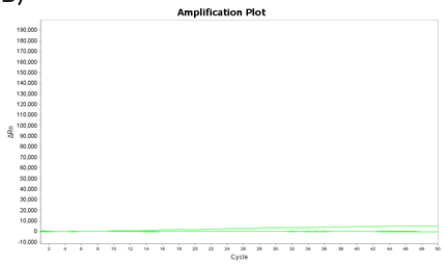
Detection of colorectal cancer-associated p53 mutation (SNP accession number: rs1042522) was performed using A) Lyo-Ready™ Genotyping Direct qPCR Stool, B) Kapa Probe Force, C) TaqPath™ and D) Type-it Fast Kits, in presence of 20 mg/mL human stool. Homozygous Allele C (red) and Allele G (blue) and heterozygous Allele C/G (green) with a NTC (black) and x for undetermined. Approximately half of all colorectal cancers show p53 gene mutations, and advanced gastric cancer treated with paclitaxel and cisplatin combination chemotherapy found that rs1042522(G;G) and (C;G) genotypes correlate with worse progression[2]. The results illustrate the ability of Lyo-Ready™ Genotyping Direct qPCR Stool to form tight clusters and to achieve accurate allelic discrimination of clinically relevant mutations directly from crude human stool samples.

Detection of Clarithromycin Resistance-Associated *H. pylori* from 3.33 mg/mL Human Stool

A)



B)



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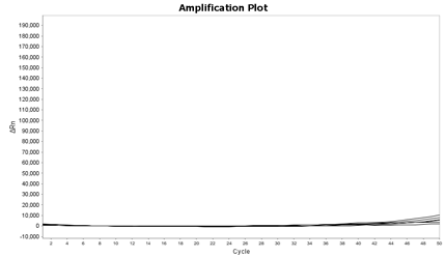
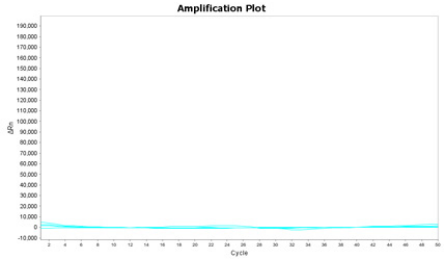


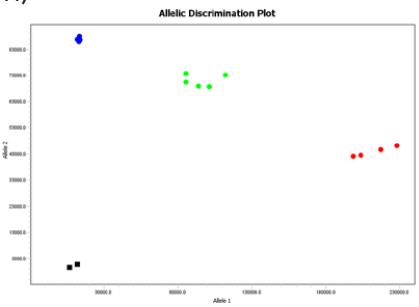
D)



Detection of clarithromycin resistance-associated *Helicobacter pylori* 23s rRNA gene A2143G mutation was performed using A) Lyo-Ready™ Genotyping Direct qPCR Stool (MDX148), B) KAPA Probe Force qPCR Mix (Roche), C) TaqPath™ ProAmp™ Multiplex Master Mix (ThermoFisher) and D) Type-it Fast SNP Probe PCR Kits (Qiagen) in presence of 3.33 mg/mL human stool. *H. pylori* treatment uses clarithromycin-based triple therapy, however rising rates of clarithromycin resistance is resulting in high failure rates. Clarithromycin resistance was observed to be most commonly due to A2143G alterations in the 23S ribosomal RNA gene[3]. Lyo-Ready™ Genotyping Direct qPCR Stool however illustrates the ability to detect the presence of low levels of the 23S ribosomal RNA gene directly from stool samples, making it an ideal fast alternative to culture which is difficult and time-consuming.

Detection of Shiga Toxin-Producing *E. coli* Strain uidA Gene Mutation from 10 mg/mL Human Stool

A)



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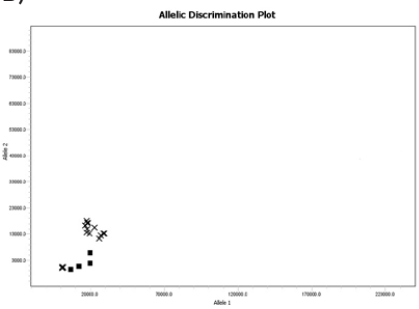

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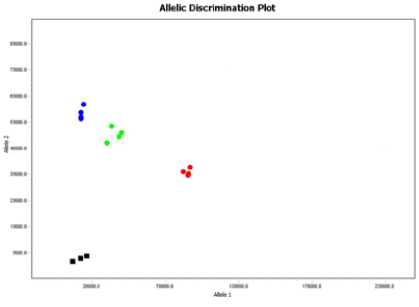
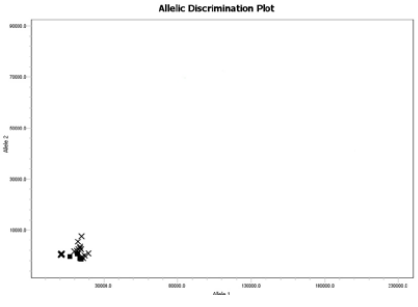


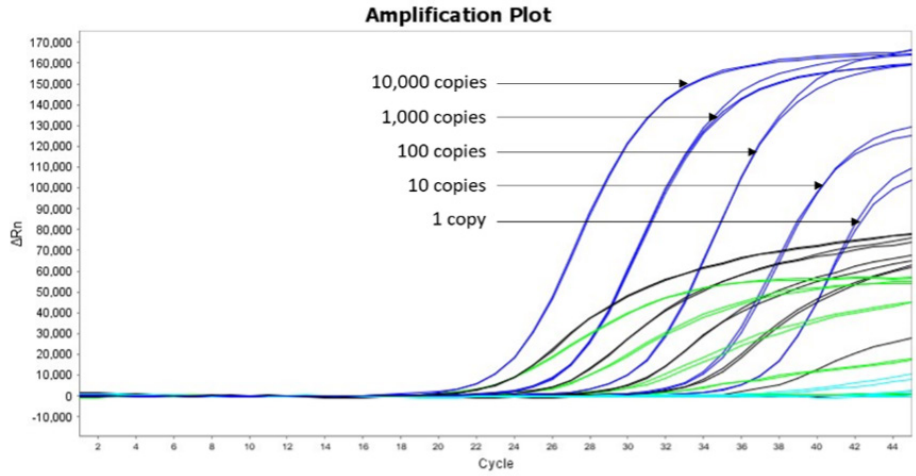
D)



Detection of shiga toxin-producing *Escherichia coli* (STEC) strains uidA gene mutation was performed using A) Lyo-Ready™ Genotyping Direct qPCR Stool (MDX148), B) KAPA Probe Force qPCR Mix (Roche), C) TaqPath™ ProAmp™ Multiplex Master Mix (ThermoFisher) and D) Type-it Fast SNP Probe PCR Kits (Qiagen) in the presence of 10 mg/mL human stool. Homozygous Allele T (red) and Allele G (blue) and heterozygous Allele T/G (green) with a NTC (black) and x for undetermined. O157:H7 STEC serotype is associated with serious illnesses in humans and even death. A highly conserved marker of O157:H7 strains is in the uidA gene which has a G residue (rather than the T residue found in wild-type *E. coli*) at position 92[4]. This mutation is difficult to detect, however, Lyo-Ready™ Genotyping Direct qPCR Stool illustrates the ability to detect the presence of low levels of the 23S ribosomal RNA gene directly from stool samples, making it an ideal fast alternative.

Allele G | Allele T | Allele T/G | NTC

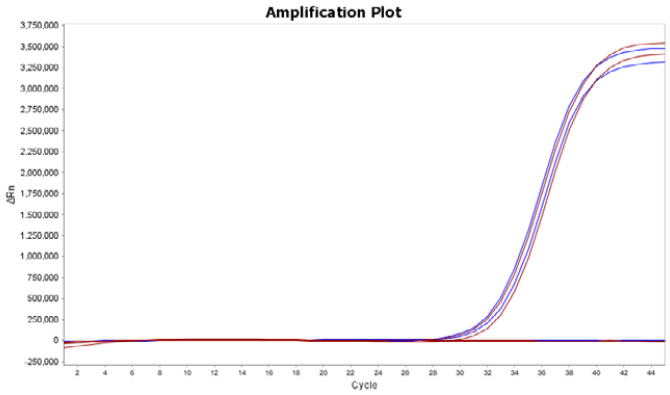
High Sensitivity and Reproducibility in Assays Detecting High or Low copies of DNA Templates in 15 mg/mL Human Stool



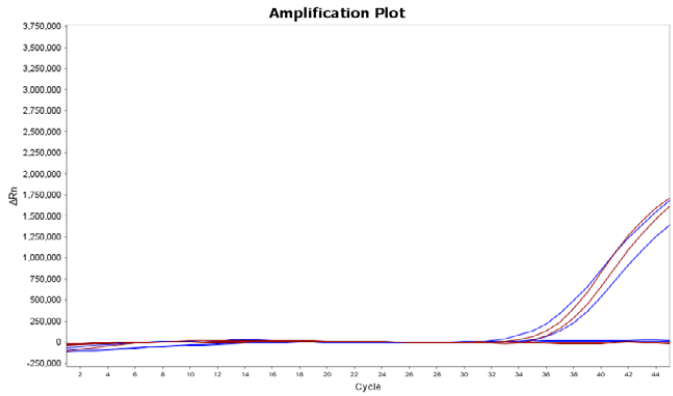
The activity of Lyo-Ready™ Genotyping Direct qPCR Stool (blue) was tested in a qPCR assay using a 10-fold serial dilution of synthetic DNA (10,000, 1000, 100, 10 and 1 copies respectively), in the presence of 15 mg/mL human stool, and compared to KAPA Probe Force qPCR Mix (green), TaqPath™ ProAmp™ Multiplex Master Mix (black) and Type-it Fast SNP Probe PCR (turquoise). The results illustrate that Lyo-Ready Genotyping Direct qPCR Stool can sensitively and reproducibly detect high or low copies of DNA templates in the presence of PCR inhibitors.

Stable Shelf Life for up to 18 months After Lyophilization

A)



B)



Lyo-Ready™ Genotyping Direct qPCR Stool was lyophilized, and the stability of the dried assays (red) was tested after 1 month at 37°C against fresh liquid mix (blue) with A) purified DNA target, and B) in the presence of 10 mg/mL human stool. The lyophilized mixes retain the same performance as the liquid mix, and the same accurate allelic discrimination where mutant allele-specific amplification is retained while WT non-specific amplification is not observed. Results suggest that Lyo-Ready™ Genotyping Direct qPCR Stool mix has a projected stability of more than 18 months at ambient temperature.

1. NCCN clinical cancer practice guideline in oncology (NCCN Guidelines) Ver.4-June 15, 2020 http://nccn.org/professionals/physician_gls/pdf/colon.pdf
2. Kim, J.G., *et al.* TP53 codon 72 polymorphism associated with prognosis in patients with advanced gastric cancer treated with paclitaxel and cisplatin. *Cancer Chemother Pharmacol.* 2009 Jul;64(2):355-60. doi: 10.1007/s00280-008-0879-3
3. Shen, J., *et al.* Formation of A2143G mutation of 23S rRNA in progression of clarithromycin-resistance in Helicobacter pylori 26695. *Microb Drug Resist.* 2005, 11: 100-106. 10.1089/mdr.2005.11.100.
4. Cebula, T.A., *et al.* Simultaneous Identification of Strains of Escherichia coli Serotype O157:H7 and Their Shiga-Like Toxin Type by Mismatch Amplification Mutation Assay-Multiplex PCR. *J. Clin. Microbiol.*, Jan. 1995, p. 248-250

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