Lyo-Ready™ Genotyping Direct DNA qPCR Urine

Enabling sensitive detection of SNPs from urine samples

Lyo-Ready™ Genotyping Direct qPCR Urine 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, non-invasive prenatal testing 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, and they play a significant role in generating genetic diversity among individuals. While not all point mutations have functional consequences, some can significantly impact gene function or protein structure and this in turn can increase or decrease an individual's risk of developing diseases such as cancer.

Genotyping from urine samples can be challenging because urine typically contains a lower concentration of DNA compared to other biological samples such as blood or saliva. In addition, other factors such as the presence of inhibitors, and the sensitivity of the genotyping method used can influence the success of a genotyping assay. However, advances in genotyping technologies, have made it possible to extract and analyze DNA from urine samples for various purposes, including:

1. **Infectious Disease Testing:** Detection of STDs, including HPV type testing, and UTIs
2. **Cancer Biomarker Detection:** Early-stage screening for cancers such as bladder cancer
3. **Non-Invasive Prenatal Testing (NIPT):** Screening assay for fetal genetic abnormalities using cell-free fetal DNA from the mother's urine
4. **Pharmacogenomics:** Identification of genetic variations that influence an individual's response to certain drugs
5. **Research & Epidemiology Studies:** Population genetics research to provide insights into the genetic basis of various traits or diseases

Meridian's new Lyo-Ready™ Genotyping Direct qPCR Urine 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 urine samples and overcomes inhibitors such as urea and nucleases 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 urine (e.g. urea, nucleases) and in transport media (such as UTM for vaginal swab)
- 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 (POC) testing.
- Compatible with a range of lyophilization protocols

PRODUCT	CAT NO.	VOLUME	REACTIONS
Lyo-Ready™ Genotyping Direct DNA qPCR Urine, 4x	MDX158	5 mL	1,000 Rxns
		50 mL	10,000 Rxns

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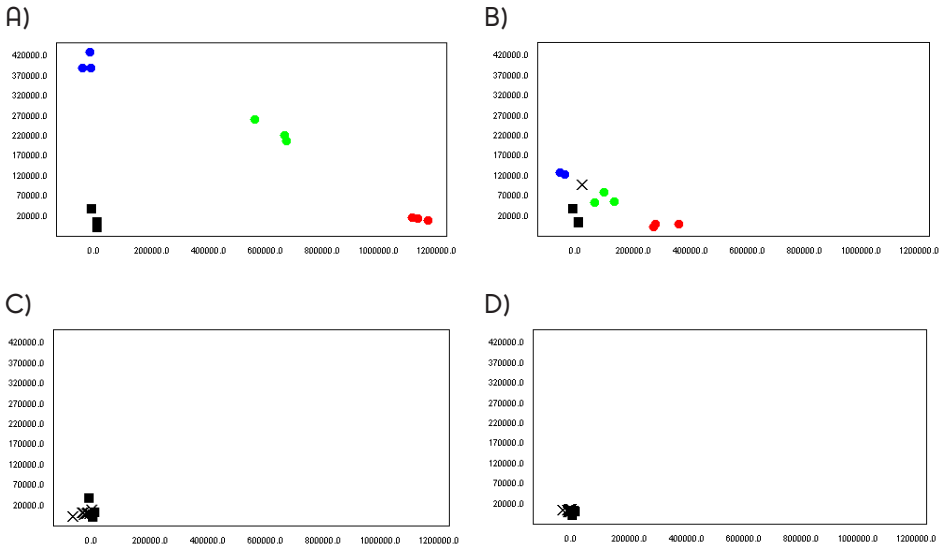
Performance Data

Lyo-Ready™ Genotyping Direct qPCR Urine 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 urine (and transport media).

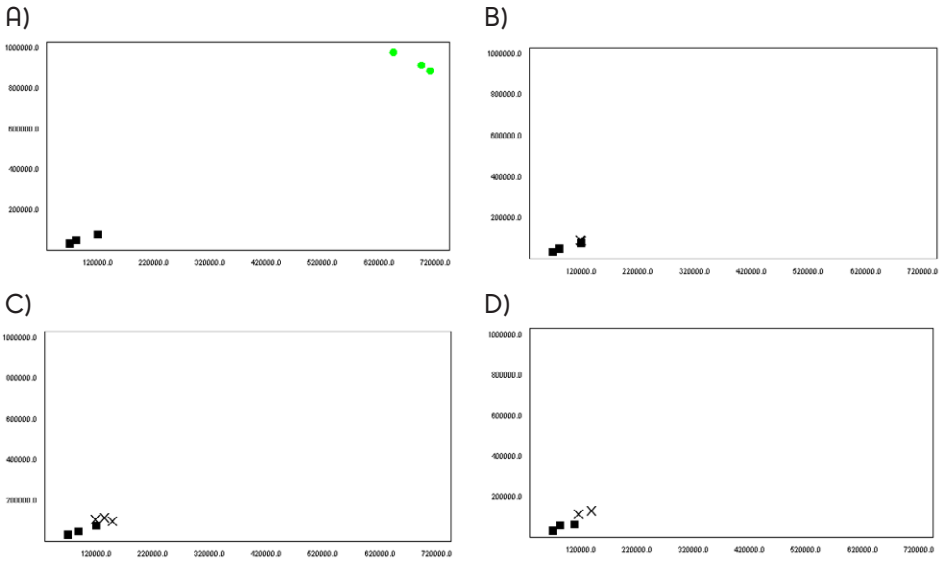
Detection of Human Papillomavirus (HPV) Type 16 from 40% Universal Transport Media (UTM)



Detection of human papillomavirus (HPV) type 16 E6-T350G mutation was performed using A) Lyo-Ready™ Genotyping Direct qPCR Urine (MDX158), 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 40% Universal Transport Media (UTM). Homozygous samples for Allele G (**blue**) and Allele T (**red**) and heterozygous samples for Allele G/T (**green**) were compared with a NTC (**black**) and x for undetermined. The presence of the 16 E6-T350G change may have biological advantages that promote cell immortalization in cervical and oropharyngeal cancer[1]. The results illustrate ability of Lyo-Ready™ Genotyping Direct qPCR Urine to form tight clustering and so accurate allelic discrimination in the presence of clinically relevant mutations directly from universal transport media.

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

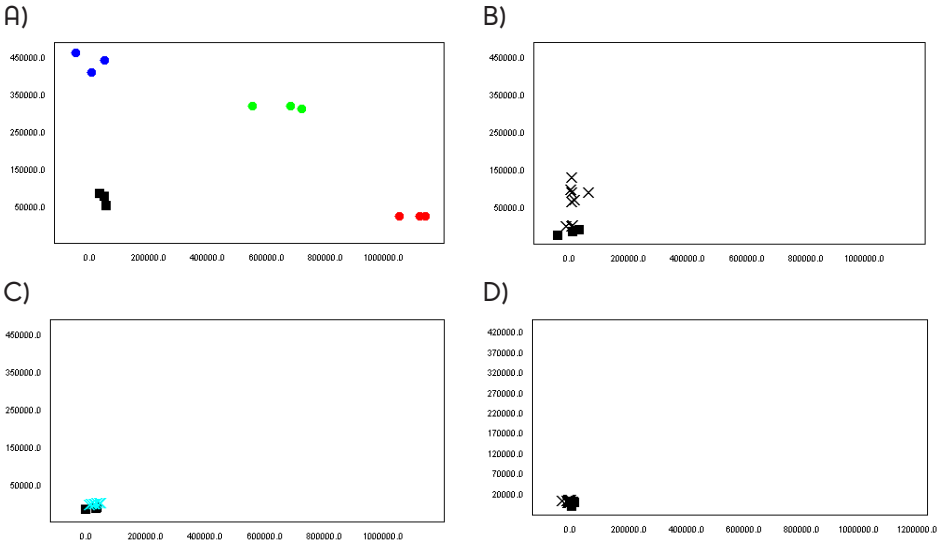
Detection of Pancreatic Cancer-associated KRAS-G12D Mutation from 10% Pooled Human Urine



Detection of pancreatic cancer-associated KRAS-G12D mutation (SNP accession number: rs121913529) from DNA of pancreatic cancer cell line SU.86.86 was performed using A/ Lyo-Ready™ Genotyping Direct qPCR Urine, B/ Kapa Probe Force, C/ TaqPath™ and D/ Type-it Fast Kits, in presence of 10% pooled human urine. Homozygous Allele A (**blue**) and Allele G (**red**) and heterozygous Allele A/G (**green**) with a NTC (**black**) and x for undetermined. The KRAS-G12D mutation is present in approximately 35% of people diagnosed with pancreatic cancer, however new studies in mice have identified a promising experimental drug that directly targets pancreatic tumors with this mutation[2]. The results illustrate the ability of Lyo-Ready™ Genotyping Direct qPCR Urine to detect and accurately discriminate cancer-associated SNPs from human cancer cells directly from crude human urine samples.

Allele A | Allele G | Allele A/G | NTC

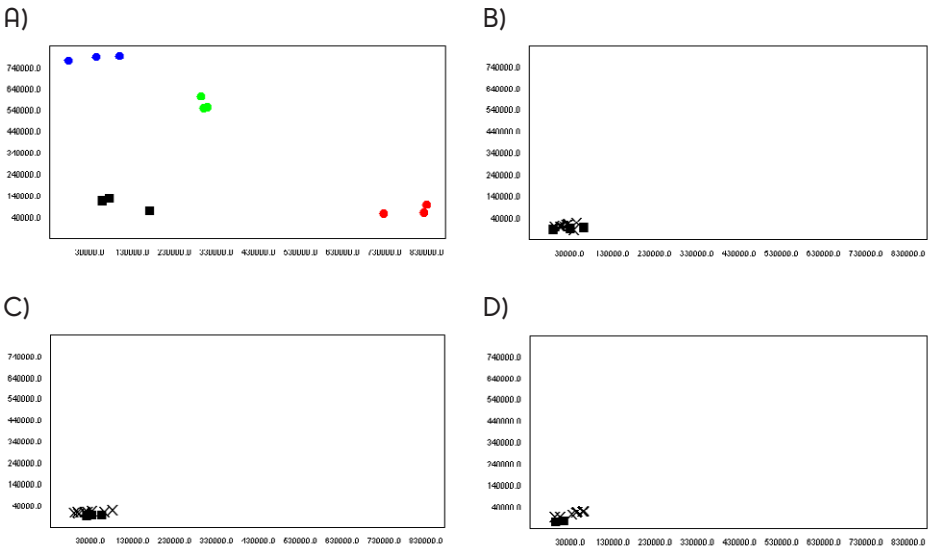
Detection of Cervical Cancer-associated p53 Mutation from 20% Pooled Human Urine



Allele G | Allele C | Allele C/G | NTC

Detection of cervical cancer-associated p53 mutation (SNP accession number: rs1042522) was performed using A/ Lyo-Ready™ Genotyping Direct qPCR Urine, B/ Kapa Probe Force, C/ TaqPath™ and D/ Type-it Fast Kits, in presence of 20% pooled human urine. Homozygous Allele G (blue) and Allele C (red) and heterozygous Allele C/G (green) with a NTC (black) and x for undetermined. Meta-analysis suggested that p53 rs1042522 C>G polymorphisms are associated with the increased risk of cervical cancer[3]. Genotyping analysis from the Lyo-Ready™ Genotyping Direct qPCR Urine Mix illustrate accurate allelic discrimination clustering in the presence of 20% urine.

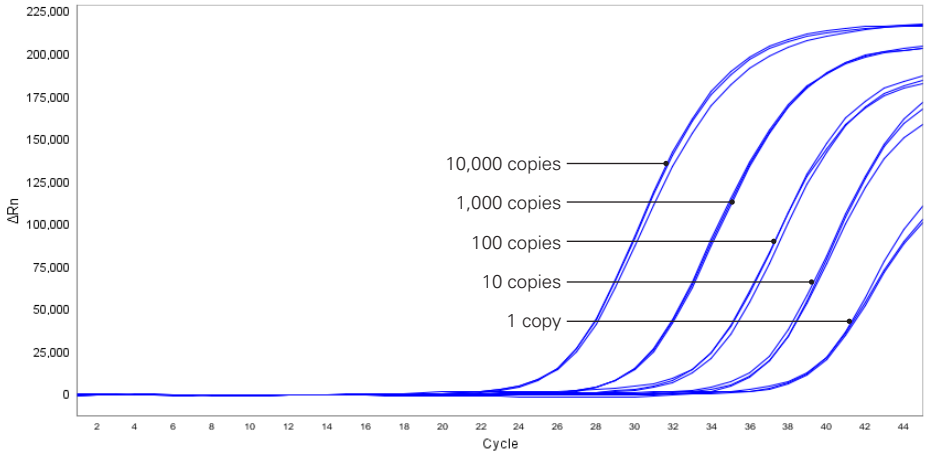
Detection of Bladder Cancer-associated PAI1 Mutation from 30% Pooled Human Urine



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

Detection of bladder cancer-associated PAI1 mutation (SNP accession number: rs7242) was performed using A/ Lyo-Ready™ Genotyping Direct qPCR Urine, B/ Kapa Probe Force, C/ TaqPath™ and D/ Type-it Fast Kits, in presence of 30% pooled human urine. Homozygous Allele G (blue) and Allele T (red) and heterozygous Allele G/T (green) with a NTC (black) and x for undetermined. Bladder cancer is one of the most common cancer types worldwide and is characterized by a high rate of recurrence, recent studies suggest SNP rs7242 increased the anti-apoptotic effect of plasminogen activator inhibitor-1 (PAI1) indicating a potentially higher risk of bladder cancer recurrence[4]. Genotyping analysis from the Lyo-Ready™ Genotyping Direct qPCR Urine Mix illustrate accurate allelic discrimination clustering in the presence of 30% urine.

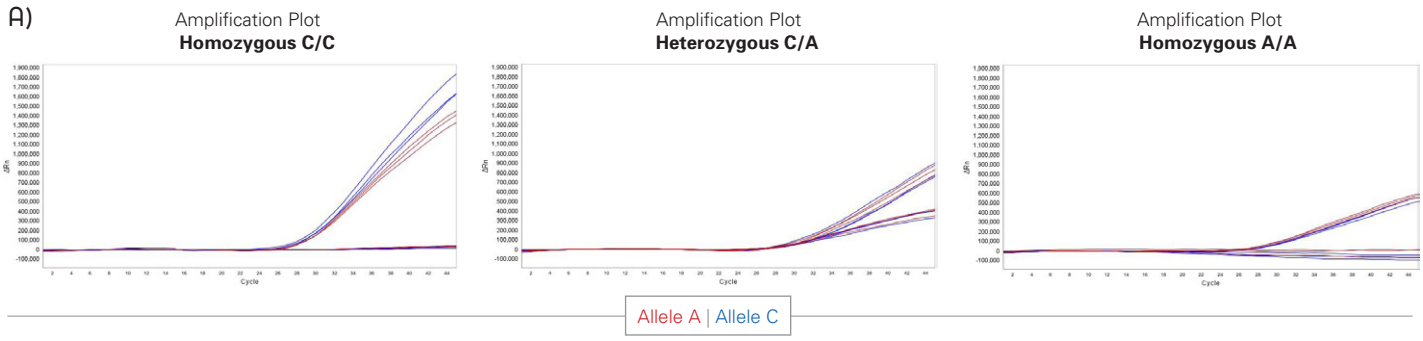
High Sensitivity and Reproducibility in Assays Detecting High or Low copies of DNA Templates in 10% Urine



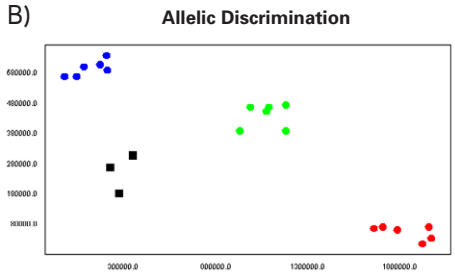
The activity of Lyo-Ready™ Genotyping Direct qPCR Urine was tested in a qPCR assay using a 10-fold serial dilution of synthetic DNA (10,000, 1000, 100, 10 and 1 copies respectively), in the presence of 10% pooled human urine. The results illustrate that Lyo-Ready™ Genotyping Direct qPCR Urine has high sensitivity and reproducibility in assays detecting high or low copies of DNA templates and in the presence of PCR inhibitors.

Stable Shelf Life for up to 18 months After Lyophilization

A)



B)



Lyo-Ready™ Genotyping Direct qPCR Urine 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 10% pooled human urine. The lyophilized mixes retained A) the same performance as the liquid mix and B) the same accurate allelic discrimination (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 Urine mix has a projected stability of more than 18 months at ambient temperature.

1. Kottaridi, C., *et al.* The T350G Variation of Human Papillomavirus 16 E6 Gene Prevails in Oropharyngeal Cancer from a Small Cohort of Greek Patients. *Viruses* 2022 14:1724. doi: 10.3390/v14081724
2. Kemp, S.B., *et al.* Efficacy of a Small-Molecule Inhibitor of KrasG12D in Immunocompetent Models of Pancreatic Cancer. *Cancer Discov.* 2023 13(2):298-311. doi: 10.1158/2159-8290.CD-22-1066.
3. Yu M., *et al.* Associations of MDM2 rs2279744 and TP53 rs1042522 polymorphisms with cervical cancer risk: A meta-analysis and systematic review. *Front Oncol*, 2022 12, 973077. doi: 10.3389/fonc.2022.973077.
4. Murakami K., *et al.* Association of SNPs in the PAI1 Gene with Disease Recurrence and Clinical Outcome in Bladder Cancer. *Int J Mol Sci.* 2023 24(5):4943. doi: 10.3390/ijms24054943.

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