CYP2C19 Genotyping HRM Kit

Catalog Number : CYP-001-0020
20 tests/kit

For Research Use Only

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Background Information

Cytochrome P450 2C19 (abbreviated CYP2C19) gene produces an enzyme which is involved in protein processing and transport in liver cells. CYP2C19 enzyme plays a role in the processing or metabolizing of at least 10 percent of commonly prescribed drugs, including a drug called clopidogrel (also known as Plavix). Clopidogrel is an antiplatelet drug that CYP2C19 enzyme activates its active form to inhibit receptor protein on the surface of platelets. CYP2C19*2 and CYP2C19*3 are most common CYP2C19 gene polymorphisms associated with clopidogrel resistance result in the production of a nonfunctional CYP2C19 enzyme that is unable to activate clopidogrel. In contrast, CYP2C19*17 polymorphism enhances antiplatelet activity of clopidogrel. CYP2C19 also play a important role in the metabolism of Omeprazole. Omeprazole is Proton pump inhibitors (PPIs) that are used in the treatment of stomach-acid related disorders. The CYP2C19*17 variant allele which is associated with increased enzyme activity may enhance PPI clearance.

FemtoPath CYP2C19 Genotyping HRM Kit is a highly specific and sensitive HRM-based test able to detect genetic variants in CYP2C19. Femtopath CYP2C19 Genotyping HRM Kit enables to identify CYP2C19*2, CYP2C19*3 and CYP2C19*17 variants. CYP2C19*2, CYP2C19*3, and CYP2C19*17, could explain most of the phenotypes related to the voriconazole metabolism and some of its pharmacokinetic singularities.

Intended Use

Identification of CYP2C19 genetic variants including CYP2C19*2, CYP2C19*3 and CYP2C19*17.

Kit Components

Vial Number	Name of Component	Description	Volume (µl)
1	CYP2C19*2 Primer Mix	Position 681 of the gene (G/A)	80
2	CYP2C19*3 Primer Mix	Position 636 of the gene (G/A)	80
3	CYP2C19*17 Primer Mix	Position -806 of the gene (C/T)	80
4	2X HRM Master Mix	qPCR reaction polymerase pre-mixture	200 x 3
5	DDH2O	Sterile H2O	1000

Additional Equipment and Reagents Not Supplied

DNA extraction reagents and equipments
Sterile, nuclease-free PCR tubes
Pipettes and appropriate pipette tips
qPCR instruments

Storage

Store at -20°C. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

DNA Preparation

Human genomic DNA must be extracted from formalin-fixed paraffin-embedded (FFPET), fine needle biopsy or pleural effusion specimens. We recommend using Qiagen DNA extraction kit (QIAamp DNA FFPE Tissue Kit, cat No. 56404) for genomic DNA extractions. The OD value of genomic DNA extractions should be measured using the spectrophotometer. Extracted genomic DNA specimens may be stored at -20 °C for long term storage.

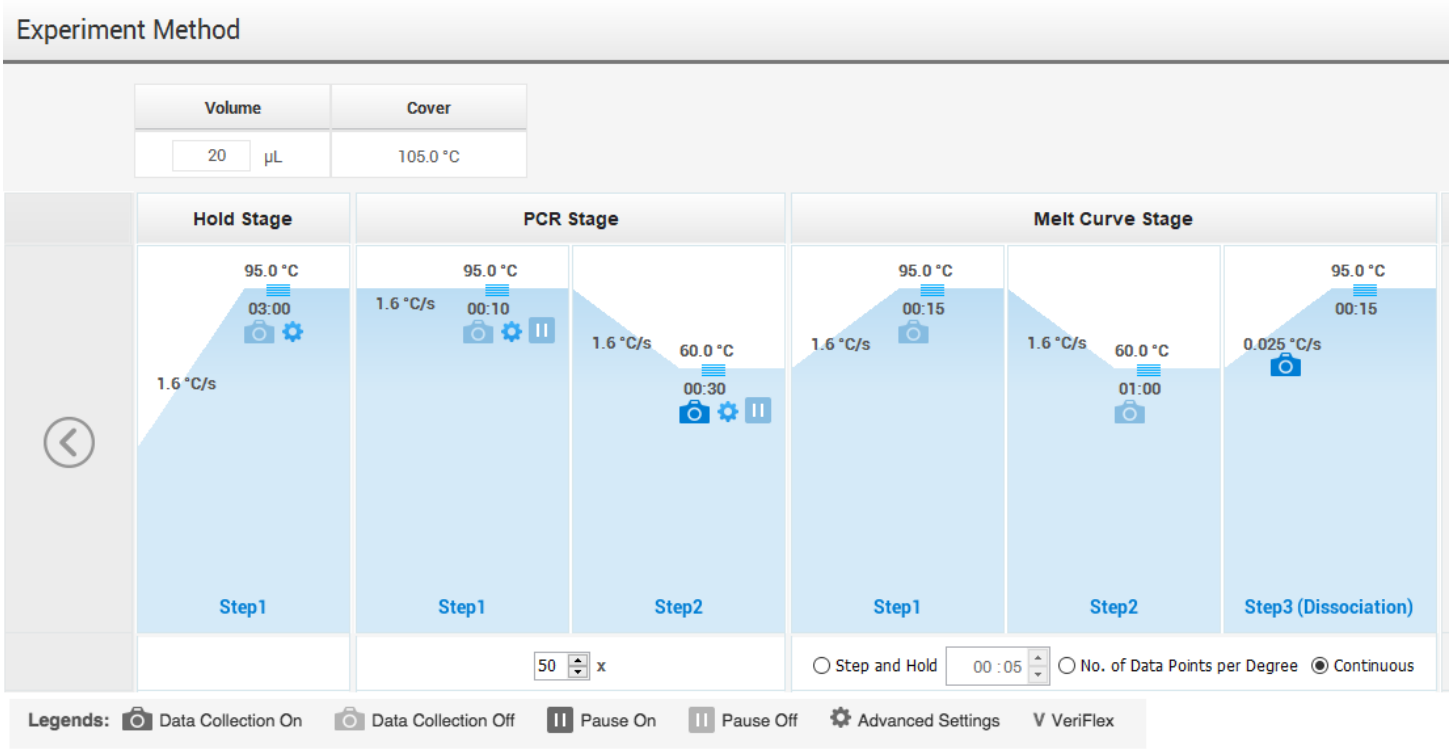
FemtoPath CYP2C19 Genotyping HRM Test Preparation

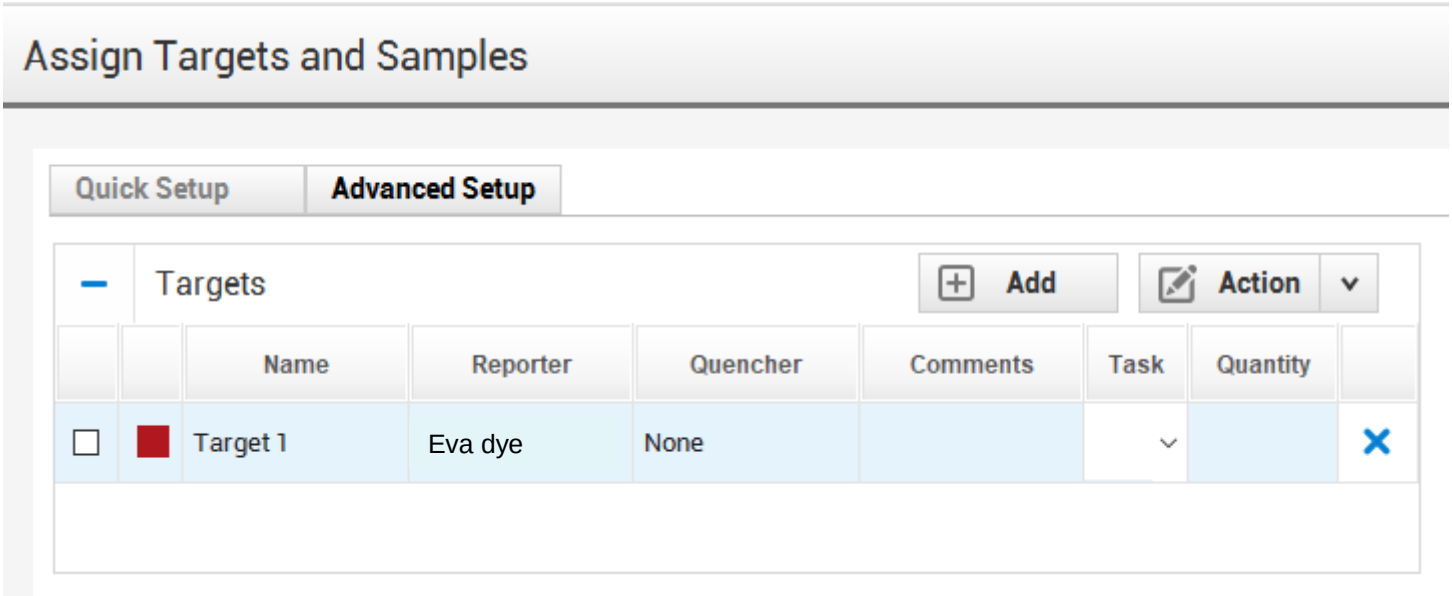
1. Thaw and centrifuge all vials in the kit.
2. Keep 2x HRM Master Mix in ice and dark to prevent enzyme degradation
3. Prepare separately qPCR Reaction Mixture by adding 10 µl 2x HRM Master Mix, 10 µl CYP2C19 Primer Mixes and 5µl Sterile H2O with a total of 19 µl mixture per reaction.
4. Add 1 µl (20~100 ng) DNA specimen into the PCR reaction mixture.

Components	Volume (µl)
DNA specimen (20~100 ng)	1
CYP2C19 Primer Mixes	4
2x HRM Master Mix	10
DDH_2O	5
Total Volume	20

QuantStudio™ 3 or 5 Real-Time PCR Cycling Condition

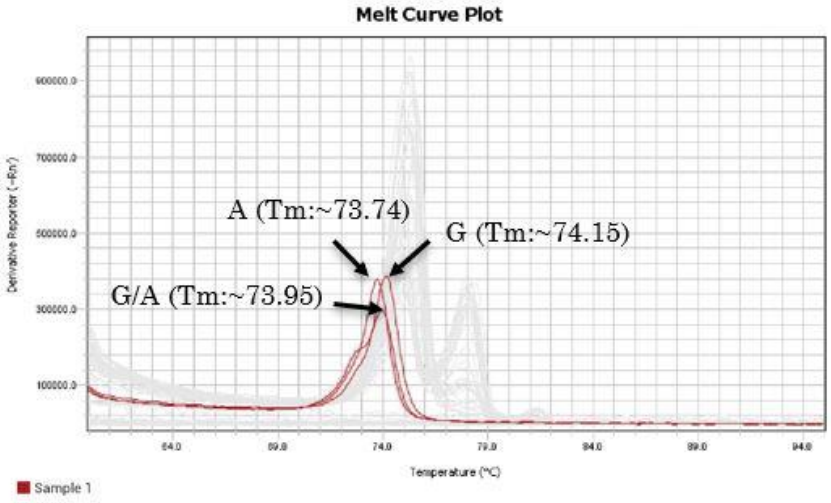
Centrifuge all PCR mixture and perform qPCR using the cycling conditions below.



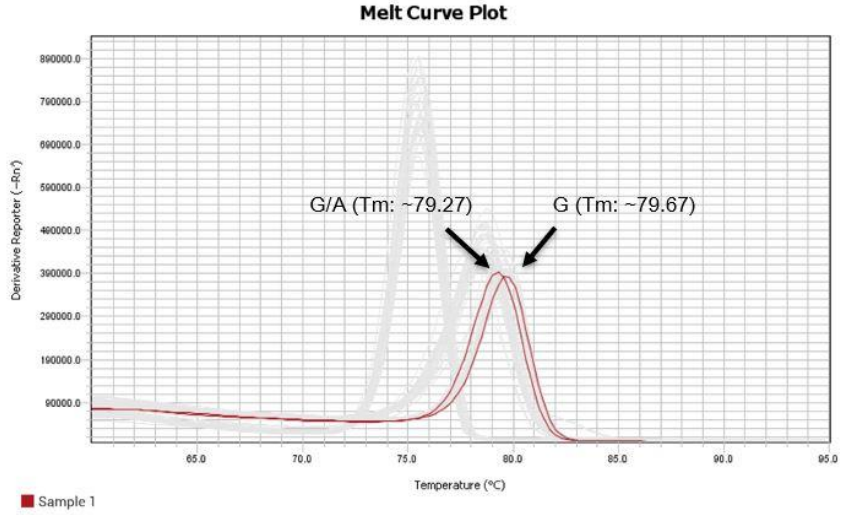


Data Analysis

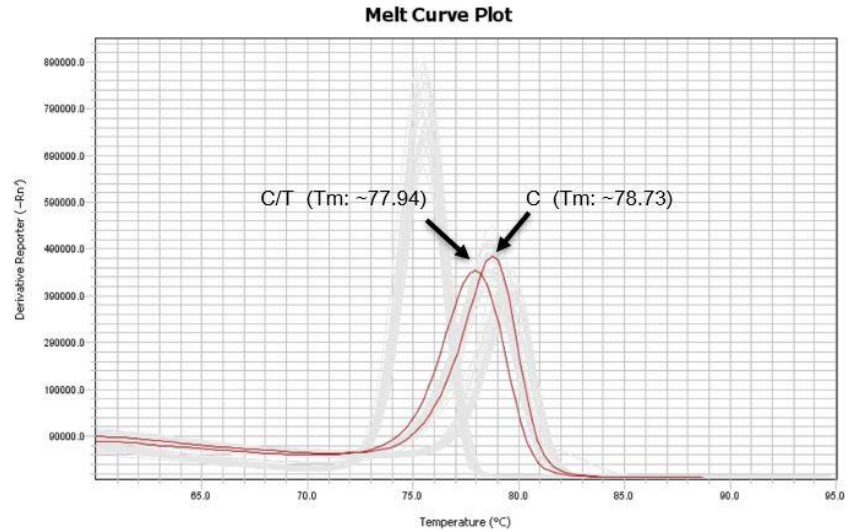
CYP2C19*2



CYP2C19*3



CYP2C19*17



Warnings and Precautions

1. Please read the instruction carefully before use.
2. Experiments should be performed under proper sterile condition with aseptic techniques.
3. Thawed thoroughly, mix the components by inverting and centrifuge briefly before use.
4. Use sterile pipette tips and dedicated pipettes to prevent exogenous DNA contamination .
5. Strictly not recommended to add other sources of reagents.

References

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7. Baldwin, R. M. et al. Increased omeprazole metabolism in carriers of the CYP2C19*17 allele; a pharmacokinetic study in healthy volunteers. Br J Clin Pharmacol 65, 767-774, doi:10.1111/j.1365-2125.2008.03104.x (2008).
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