CLOPIDOGREL REAL TIME PCR KIT (3 MUTATIONS)

Cat. No: 113R-20-03

PRODUCT DESCRIPTION

Clopidogrel is a widely used antiplatelet agent to treat and prevent a variety of atherothrombotic diseases. Research has implicated genetic variations in the CYP2C19 isozyme as at least partly responsible for the variable antiplatelet response seen with clopidogrel. Studies have shown that patients possessing genetic variants of the CYP2C19 isozyme may be at increased risk of adverse cardiovascular events due to impaired clopidogrel efficacy, although this has not been definitively demonstrated. Kit detects 636 G>A (*3), 681 G>A (*2) and -806 C>T (*17) mutations in CYP2C19 gene.

PRINCIPLE OF THE SYSTEM

Test uses 5' Nuclease Assay. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencer dye only when the probe hybridizes perfectly to the target DNA. This cleavage results in the fluorescent signal which is monitored by Real-Time PCR detection system. An increase in the fluorescent signal (CT) is proportional to the amount of the specific PCR product.

PRODUCT SPECIFICATION

Each isolated DNA should be tested with wild type and mutant real time pcr mastermixes. The kit provides reagents in a ready-to-use mastermix format which has been specifically adapted for 5' nuclease PCR using SNP analyses. The test system is designed to use with sequence specific primers and probe.

The fluorescence of mutation analysis is FAM, Texas RED and HEX/JOE. Also each mastermix contains an internal control labelled with CY5 dye. Mutations and related dyes can be seen in Table 1.

SYSTEM CONTENTS

Reagents	20 rxns
• Clopidogrel Master Mix 1	400 µl
• Clopidogrel Master Mix 2	400 µl
• Control DNA	75 µl

DNA EXTRACTION

Blood samples should be collected in appropriate sterile EDTA tubes and can be stored at +4°C up to one month. For more than one month specimen should be stored at -20°C. It is advised to gently mix the tube (with EDTA) after collection of blood to avoid coagulation. Our system optimized according to SNPure Blood® and MN NucleoSpin ® Blood. It is advised to elute DNA with **150 µl elution buffer** for better results.

MUTATION / DYE TABLE

Table 1 : Tubes – mutations - dyes.

Tubes	Mutations	Dyes
Mix 1	636 Wild-Type (G)	FAM
	-806 Wild-Type (C)	JOE / HEX
	681 Wild-Type (G)	Texas Red
	Internal Control	CY5
Mix 2	636 Mutant (A)	FAM
	-806 Mutant (T)	JOE / HEX
	681 Mutant (A)	Texas Red
	Internal Control	CY5

PROCEDURE

- Different tubes should be prepared for each mix.
- Before starting work, mix the mastermixes gently by pipetting
- For each sample, pipet **20 µl mastermix*** with micropipets of sterile filter tips to each optical white strips or tubes.
- Add **5 µl (~10-100 ng) DNA** into each tube**.**
- Run with the programme shown below.

Master mixes include HotStart Taq DNA Polymerase.

PCR PROGRAMME

95 °C	3 Min.	Holding
95 °C	15 Sec.	30 Cycles
60 °C	1 Min.	

Fluorescent dyes are FAM, HEX/JOE, Texas Red and CY5.

If you use;

- ABI Prism® system, please choose **"none"** as passive reference.

This system can be used with;

Bio-Rad CFX96

ABI Prism ® 7500/7500 Fast

DATA ANALYSIS

After the run is completed data are analysed using the software with FAM, HEX (JOE), Texas Red and CY5 dyes. Please see table 2 for genotyping. The below results were studied with Bio-RAD CFX96.

Table 2 : CYP2C19 Functional Status and Phenotypes

CYP2C19 Functional Status and Phenotypes *		
636 Wild Type (G) - 681 Wild Type (G) - 806 Wild Type(C)	*1	Normal function alleles
681 Mutant (A)	*2	No function allele
636 Mutant (A)	*3	No function allele
806 Mutant (T)	*17	Increased function allele

Metabolism	Genotype	For Example;
CYP2C19 Ultrarapid Metabolizer	An individual carrying 2 increased function alleles	*17/*17
CYP2C19 Rapid Metabolizer	An individual carrying one normal function allele and one increased function allele	*1/*17
CYP2C19 Normal Metabolizer	An individual carrying 2 normal function alleles	*1/*1
CYP2C19 Intermediate Metabolizer	An individual carrying one normal function allele and one no function allele or one no function allele and one increased function allele	*1/*2 *2/*17 *1/*3 *3/*17
CYP2C19 Poor Metabolizer	An individual carrying 2 no function alleles	*2/*2 *2/*3 *3/*3

* *The Clinical Pharmacogenetics Implementation Consortium (CPIC)*

CYP2C19 metabolizer status frequencies are based on average multi-ethnic frequencies.

*The predicted metabolizer phenotype for the *2/*17 genotype is a provisional classification. The currently available evidence indicates that the CYP2C19*17 increased function allele is unable to completely compensate for the CYP2C19*2 non-functional allele.*

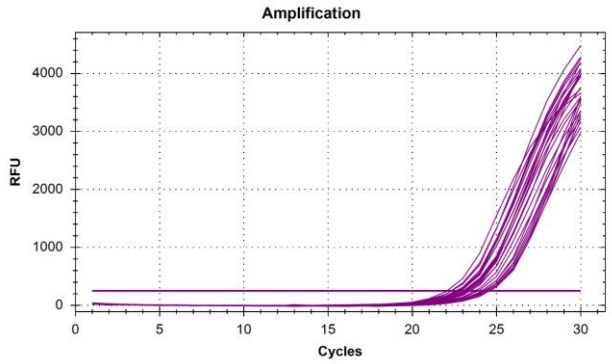

Figure 1: Internal Control plots – CY5 Dye

Internal control amplification plots must be seen in all wells except NTC and has been labelled with CY5 dye. The CT value of internal controls should be **21 ≤ X ≤ 26**.

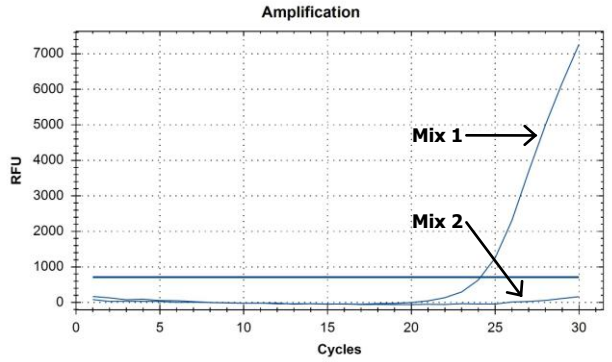

Figure 2: 636 Homozygous Wild Type – FAM Dye

Amplification plots of mutations can be analysed by related dye*. The CT value should be between **21 ≤ CT ≤ 26**. These values are optimised according to the SNPure® Blood DNA Isolation Kit and MN NucleoSpin ® Blood DNA Isolation Kit. CT values may vary ±2/3 cycle according to the DNA isolation protocol.

- Homozygous wild-type sample gives amplification signal only with wild-type mastermix.
- Heterozygous sample gives amplification signal both with wild-type and mutant mastermixes.
- Homozygous mutant sample gives amplification signal only with mutant mastermix.

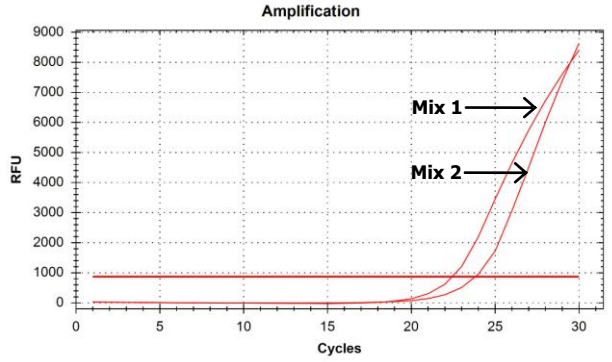

Figure 3: 681 Heterozygous Sample – Texas Red Dye

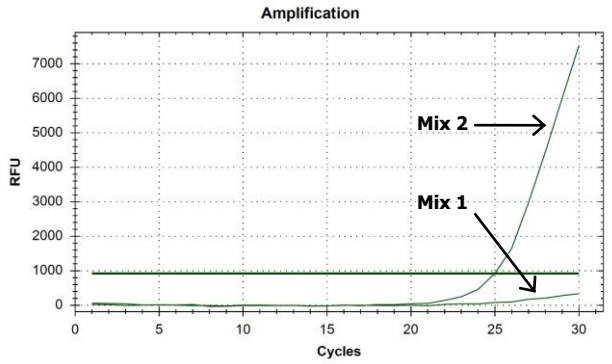

Figure 4: -806 Homozygous Mutant Sample – HEX Dye

- The diffrence of the CT value wild-type and mutant amplification plots should be **≤3** for heterozygous mutant sample. It is **4 ≤ CT ≤6**, test should be repeated.

**Please check tubes / mutations / dyes table (table 1).*

TROUBLE SHOOTING

If internal control doesn't work,

- Absence of DNA
- Sample is containing DNA inhibitor(s)

If plots start late,

- DNA quality is not good.
- The amount of DNA is not enough.
- Sample is containing parcial DNA inhibitor(s)

Please contact us for your questions. tech@snp.com.tr

CAUTIONS

- All reagents should be stored at suitable conditions.
- Do not use the PCR mastermixes forgotten at room temperature.
- Thaw PCR mastermix at room temperature and slowly mix by inverting before use.
- Shelf-life of PCR mastermix is 12 months. Please check the manufacturing data before use.
- Only use in vitro diagnostics.

STORAGE

- All reagents should be stored at – 20 °C and dark.
- All reagents can be used until the expiration date on the box label.
- Repeated thawing and freezing (>4X) should be avoided, as this may reduce the sensitivity of the assay.

REFERENCES

1. Goswami S, Cheng-Lai A, Nawarskas J. "Clopidogrel and genetic testing: is it necessary for everyone?" Cardiol Rev. 2012 Mar-Apr;20(2):96-100
2. Lee J.H., Ahn S.G., Lee J.W., Youn Y.J., et al. Switching from prasugrel to clopidogrel based on Cytochrome P450 2C19 genotyping in East Asian patients stabilized after acute myocardial infarction. Platelets. 2016 Jun;27(4):301–7