HEMOCHROMATOSIS REAL TIME PCR KIT (2 MUTATIONS)

Cat. No: 12R-10-02

PRODUCT DESCRIPTION

Hereditary hemochromatosis is an inherited disorder that increases the amount of iron, body absorbs from the gut. Symptoms are caused by this excess iron being deposited in multiple organs of the body. Most commonly, excess iron in the liver causes cirrhosis, which may develop into liver cancer. Iron deposits in the pancreas can result in diabetes. Similarly, excess iron stores can cause cardiomyopathy, pigmentation of the skin, and arthritis. Many mutations in the body's iron transport system can cause hemochromatosis; however, most cases are caused by mutations in the HFE gene. This is located on chromosome 6, and one mutation leads to the substitution of the 282nd amino acid Cysteine becomes tyrosine, therefore the mutation is called C282Y.

PRINCIPLE OF THE SYSTEM

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 patented SNP analyses. The test system is designed for use with sequence specific primers and probe.

The fluorescence of mutation analysis is FAM. Also each mastermix contains an internal control labelled with HEX/JOE dye.

SYSTEM CONTENTS

Reagents	20 rxns
• H63D Wild type PCR mastermix	400 µl
• H63D Mutant PCR mastermix	400 µl
• C282Y Wild type PCR mastermix	400 µl
• C282Y Mutant PCR mastermix	400 µl
• Control DNA	20 µl

* Control DNA is a synthetic plasmid containing some of the mutation regions. Expected results for synthetic control DNA should be H63D, C282Y Heterozygote. Since to Control DNA is a synthetic plasmid, amplification plots of synthetic control DNA may appear slightly different from the sample DNA.

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 (>3X) should be avoided, as this may reduce the sensitivity of the assay.

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 MN NucleoSpin ® Blood. It is advised to elute DNA with **150 µl elution buffer** for better results.

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 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 and HEX/JOE

If you use;

- ABI Prism® system, please choose **"none"** as passive reference and quencher.
- Mic qPCR Cycler, please adjust gain settings, **"Green Auto Gain"** to **20** and **"Yellow Auto Gain"** to **10.**

This system can use with;

Bio-Rad CFX96

ABI Prism ® 7500/7500 Fast

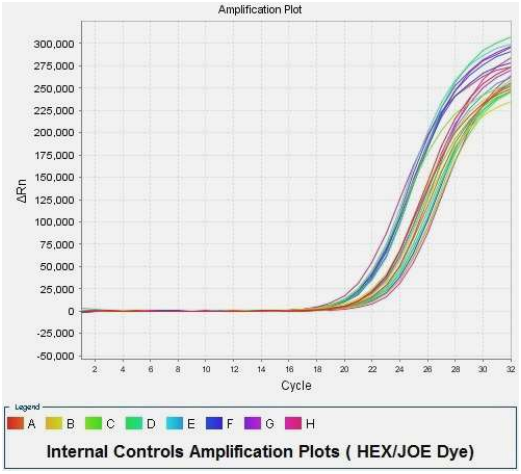
Roche LightCycler® 480 System

Rotor Gene Q

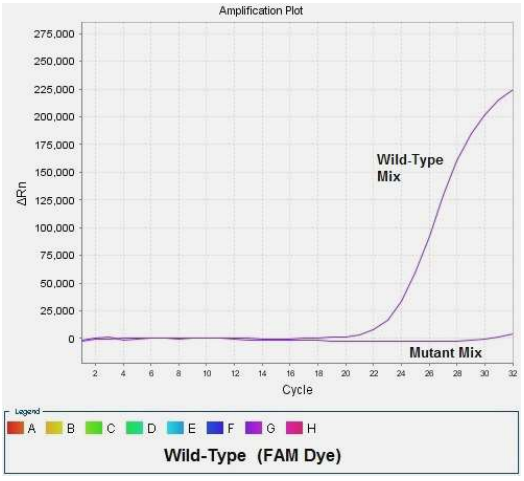
Mic qPCR Cycler

DATA ANALYSIS

After the run is completed data are analysed using the software with HEX (JOE) and FAM dyes. The below results were studied with ABI7500.

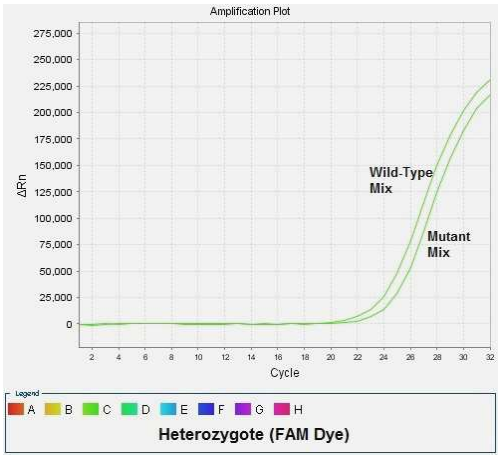


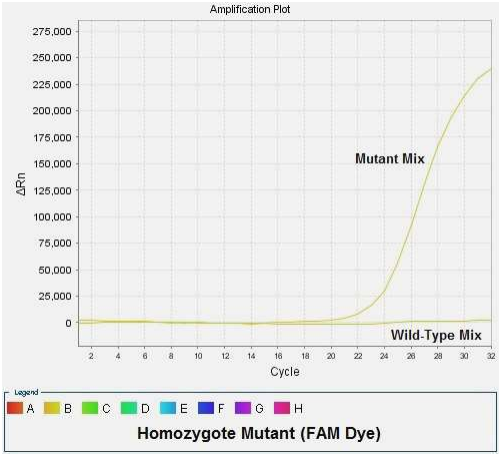
Internal control amplification plots must be seen in all wells except NTC and has been labelled with HEX (JOE) dye. The CT value of internal controls should be **22 ≤ X ≤ 26**.



Amplification plots of mutations can be analysed by FMA 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.

- Homozygote wild-type sample gives amplification signal only with wild-type mastermix.
- Heterozygote sample gives amplification signal both with wild-type and mutant mastermixes.
- Homozygote mutant sample gives amplification signal only with mutant mastermix.
- The diffrence of the CT value wild-type and mutant amplification plots should be **≤3** for heterozygote mutant sample. It is **4 ≤ CT ≤6**, test should be repeated.





TROUBLE SHOOTING

If internal control doesn't work,

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

If plots start late,

Compare positive control and sample. If there is no problem in positive control,

- DNA quality is not good.
- The amount of DNA is not enough.

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.