OCTOBIO

OCTO Health RT-PCR Hemochromatosis Mutation Kit

Catalog #	Description
RT-HFE-25	Real-Time PCR Hemochromatosis Mutation Kit, 25 reactions
RT-HFE-50	Real-Time PCR Hemochromatosis Mutation Kit, 50 reactions

IVD

CE

Introduction

OCTO Health RT-PCR Hemochromatosis Mutation Kit is an in vitro diagnostic assay intended for the qualitative detection of clinically significant mutations associated with hereditary hemochromatosis from human genomic DNA using Real-Time PCR technology.

The assay is designed for the detection of major mutations in the HFE gene, which are associated with increased susceptibility to hereditary hemochromatosis, an autosomal recessive disorder characterized by abnormal iron absorption and progressive iron overload. Table below displays the tube numbers where the parameters are located and which emission channels will be used.

TUBE	GENE	MUTATION	RS #	EMISSION CHANNEL
1	HFE	C282Y	rs1800562	ROX
	HFE	H63D	rs1799945	CY5
	HFE	S65C	rs1800730	CY5

Kit Contents

	CONTENT	25 REACTIONS	50 REACTIONS
1	Master Mix 1	450 µl	900 µl
2	Control Mix*	40 µl	80 µl

*The Control Mix contains synthetic wild-type and mutant sequences for each parameter listed in the table. During melt-curve analysis all parameters should display heterozygous profiles.

Required Equipment and Materials:

- Compatible RT-PCR Devices*

Biorad CFX96 and Opus, Qiagene Rotorgene, Quantstudio Real-Time PCR Systems, Roche LightCycler 480 & 96, Mic qPCR Cycler, Corbett Realtime PCR, Slan Realtime PCR, Molarray MA6000, Himedia Insta Q96, Q48 and Q48M

- DNA Isolation (Manual or automated system)
- Vortex and Centrifuge
- Pipette Set (0.5-10µl and 10-100µl)
- Sterile Pipette Tips
- Cold Block
- PCR tubes or plates compatible with the respective Real-Time PCR Device
- +4°C Refrigerator
- Sterile Gloves

* *Please contact for other compatible devices*

Storage Conditions

- All components of the kit should be stored at -20°C until the expiration date.
- Kit components should undergo freeze-thaw cycles a maximum of 5 times. If more than 5 freeze-thaw cycles are anticipated, it is necessary to divide the kit components into smaller volumes for storage.
- Negative Control included in the kit should be stored at 2-8 °C after the initial thawing.

Experimental Preparation

Preparation of Materials:

- Materials stored at -20°C should be thawed on ice or cooling rack.
- Once thawed, liquid materials should be mixed well by pipetting.

Preparation of Reactions:

1. Preparation of 1 tube is required for each sample. Reaction volumes are specified in the table. The same tubes should be prepared for the NTC.

Content	Amount (µl)
PCR Master Mix	18
DNA Isolate / NTC	2
TOTAL	20

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Real – Time PCR Protocol

Cycle	Temperature (°C)	Time (Seconds)	Fluorescent Dye
1	95 °C	300 s	
50	95 °C	10 s	
	60 °C	45 s	ROX, CY5
1	95 °C	180 s	
	55 °C	60 s	
Melt Analysis			
1	35 °C	1 s	
Initial Temp. (°C)	35 °C	**Ramp Rate (°C /sec)**	0.3
End Temperature	85 °C	**Hold (sec)**	1 s

Data Analysis – Interpretation of Results

TUBE 1				
C282Y	**Normal (66°C)**	**Mutant (60°C)**	**Result**	**Emission Channel**
	+	-	Normal	ROX
	-	+	Hom. Mutant	
	+	+	Heterozygous	
	Normal (60°C)	**Mutant (66°C)**	**Result**	**Emission Channel**
H63D	+	-	Normal	CY5
	-	+	Hom. Mutant	
	+	+	Heterozygous	
	Normal (60°C)	**Mutant (54°C)**	**Result**	**Emission Channel**
S65C	+	-	Normal	CY5
	-	+	Hom. Mutant	
	+	+	Heterozygous	

- After the Real-Time PCR stage, analysis begins by selecting the Melt Analysis option.
- Each tube must be analyzed separately.
- The analysis should be performed based on the presence or absence of peaks at the specified temperatures for each parameter.
- OctoClario™ Analysis Software can be used for automated analysis. Refer to the Software User Guide for program details.

Troubleshooting

1. If no melting peaks are observed in both controls and samples,
   - *Possible causes:* incomplete homogenization of the master mix; annealing/extension temperature set too high; insufficient or degraded template DNA.
   - *Solutions:* pipette and briefly centrifuge each reagent tube before setup; verify thermal-cycler temperatures strictly match IFU; repeat DNA extraction and check the quality of extracted DNA by fluorometry.
2. If control or sample melt-curve peaks are markedly lower in fluorescence than expected,
   - *Possible causes:* dye depletion due to ambient light exposure or prolonged room-temperature storage; expired or improperly stored reagents.
   - *Solutions:* protect master mix and reaction plates from light and keep on ice until use; check kit lot's expiration and storage logs; replace with a fresh kit if needed.
3. If melt peaks appear at temperatures outside the ±1 °C window of the expected T_m,
   - *Possible causes:* incorrect ramp rate or hold time during melt acquisition; nonspecific amplicons (e.g. primer-dimers).
   - *Solutions:* confirm melt-curve program parameters exactly match IFU.
4. If melt-curve derivative baselines are noisy or drift upward,
   - *Possible causes:* insufficient calibration of the instrument's optical modules; wells at plate edges subject to temperature lag.
   - *Solutions:* perform calibration with manufacturer's reference plate; avoid using outermost wells for critical controls or include a software-specified "edge-correction."
5. If sample genotyping calls fail QC despite clean control results,
   - *Possible causes:* sample inhibitors (e.g. heme, guanidine) carried over from extraction; pipetting errors.
   - *Solutions:* repeat extraction using a spin-column kit with an inhibitor-removal step; employ multichannel pipette with filtered tips and include replicate wells.
6. If unexpected peaks appear in the no-template control (NTC),
   - *Possible causes:* airborne DNA contamination.
   - *Solutions:* enforce unidirectional workflow (pre-PCR vs. post-PCR areas).

Tip: Always consult your instrument's maintenance log and ensure that filter sets and heating blocks are within specification before attributing anomalies to the chemistry.

Warnings and Precautions

This test is for in vitro diagnostic purposes only and should be used strictly in accordance with the instructions provided by trained personnel. Clinical samples should be considered as materials potentially carrying an infection risk and should be prepared in a laminar flow cabinet.

• Do not use expired kit. • Avoid repeated freezing and thawing of reagents as it may reduce the sensitivity of the test. • Ensure that reagents are thawed before use, then gently mix the tubes and centrifuge briefly. • Prepare the reaction mixture quickly on ice or a cold block. • Establish two separate work areas, one for isolation and the other for PCR. • Avoid transferring pipettes, tubes, and other working materials between the work areas. • Always use filtered sterile pipette tips. • Use separate lab coats and gloves for each work area.

Symbols Used

The following symbols used on labels and packaging of this product conform to the harmonized standard **TS EN ISO 15223-1**



:Number of Reactions



: Expiration Date



: Lot Number



: Reference Number



: Temperature Limit



: Refer to the User Manual



: Manufacturer



: Keep Away From Direct Sunlight

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