OCTOBIO

# OCTO Health RT-PCR Cardiovascular Disease 6in2 Kit

| Catalog # | Description |
|---|---|
| RT-CVD-6-2-25 | Real-Time PCR Cardiovascular Disease 6in2 Kit, 25 reactions |
| RT-CVD-6-2-50 | Real-Time PCR Cardiovascular Disease 6in2 Kit, 50 reactions |

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## Introduction

OCTO Health REAL TIME PCR Cardiovascular Disease 6in2 Kit is capable of diagnosing a total of six mutations in Factor V, Prothrombin, MTHFR, PAI and Factor XIII genes in two tubes. 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 | Factor V LEIDEN | G1691A | RS6025 | FAM |
| | Prothrombin | G20210A | RS1799963 | HEX |
| | MTHFR | C677T | RS1801133 | ROX |
| | MTHFR | A1298C | RS1801131 | CY5 |
| 2 | PAI | 4G/5G | RS1799762 | CY5 |
| | Factor XIII | V34L | RS5985 | ROX |

## Kit Contents

| | CONTENT | 25 REACTIONS | 50 REACTIONS |
|---|---|---|---|
| 1 | Master Mix 1 | 450 µl | 900 µl |
| 2 | Master Mix 2 | 450 µl | 900 µl |
| 3 | Control Mix 1* | 30 µl | 60 µl |
| 4 | Control Mix 2* | 30 µl | 60 µl |

*The Control Mixes contains synthetic wild-type and mutant sequences for each parameter listed in the table. During melt-curve analysis all parameters should display heterozygous profiles. Control Mix 1 should be used for Master Mix 1 and Control Mix 2 should be used for Master Mix 2.

## 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 2 tubes 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 | FAM, HEX, 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 | | | | |
|---|---|---|---|---|
| Factor V LEIDEN (G1691A) | Normal (68°C) | Mutant (64°C) | Result | Emission Channel |
| | + | - | Normal | FAM |
| | - | + | Hom. Mutant | |
| | + | + | Heterozygous | |
| | Normal (70°C) | Mutant (66°C) | Result | Emission Channel |
| Prothrombin Factor II (G20210A) | + | - | Normal | HEX |
| | - | + | Hom. Mutant | |
| | + | + | Heterozygous | |
| | Normal (69°C) | Mutant (63°C) | Result | Emission Channel |
| MTHFR (C677T) | + | - | Normal | ROX |
| | - | + | Hom. Mutant | |
| | + | + | Heterozygous | |
| | Normal (63°C) | Mutant (70°C) | Result | Emission Channel |
| MTHFR (A1298C) | + | - | Normal | CY5 |
| | - | + | Hom. Mutant | |
| | + | + | Heterozygous | |

| TUBE 2 | | | | |
|---|---|---|---|---|
| Factor XIII (V34L) | Normal (71°C) | Mutant (66°C) | Result | Emission Channel |
| | + | - | Normal | ROX |
| | - | + | Hom. Mutant | |
| | + | + | Heterozygous | |
| | 4G (70°C) | 5G (67°C) | Result | Emission Channel |
| PAI (4G/5G) | + | - | 4G-4G | CY5 |
| | - | + | 5G-5G | |
| | + | + | 4G—5G | |

- 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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