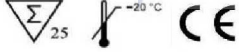


Mitochondrion DNA site 11778 Mutation Real Time PCR Kit User Manual

LT027200DG

For use with ABI Prism® 7000/7300/7500/7900/Step One Plus, iCycler iQ™4/iQ™5, Smart Cycler II, Bio-Rad CFX 96; Rotor Gene™6000; Mx3000P/3005P; MJ-Option2/Chromo4; LightCycler® 480 Instrument

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1. Intended Use

Mitochondrion DNA site 11778 Mutation Real Time PCR Kit is used for the detection of Mitochondrion DNA site 11778 Mutation in whole blood sample in the real time PCR systems.

2. Principle of Real-Time PCR

The principle of the real-time detection is based on the fluorogenic 5' nuclease assay. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities in real-time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

3. Product Description

Mitochondrion DNA 11778A mutation causes an arginine to histidine change in amino acid 340 of NADH dehydrogenase 4 (ND4), a member of the respiratory chain complex I. Studies of the influence of this mutation on respiration capacity, enzyme activity and expression level were carried out on lymphoblasts and cybrids (cells resulting from fusion of rho0 cells lacking mitochondrial DNA and enucleated cells possessing mtDNA). They showed a significant decrease in respiration of the mutated cells and slight reduction in complex I activity. Reduction in growth rate on galactose was also observed. The expression pattern of mitochondrial proteins appeared to be unchanged.

Mitochondrion DNA site 11778 Mutation Real Time PCR Kit contains a specific ready-to-use system for the detection of the Mitochondrion DNA site G11778A Mutation by PCR (polymerase chain reaction) in the real-time PCR system. One for wild type which labeled by HEX/VIC/JOE, another for mutant type which labeled by FAM. The master contains reagents and enzymes for the specific amplification of the Mitochondrion DNA site 11778 Mutation gene DNA. Fluorescence is emitted and measured by the real time systems' optical unit during PCR. There are two probes in reaction Mix. DNA extraction buffer is available in the kit and blood samples are used for DNA extraction.

4. Kit Contents

Ref.	Type of Reagent	Presentation	25rxns
1	DNA Extraction Buffer	1 vial, 1.8ml	
2	mtDNA 11778 Mutation Reaction Mix	1 vial, 950µl	
3	PCR Enzyme Mix	1 vial, 12µl	
4	Molecular Grade Water	1 vial, 400µl	
5	mtDNA 11778 Wild type Positive Control	1 vial, 30µl	
6	mtDNA 11778 Mutant type Positive Control	1 vial, 30µl	

Analysis sensitivity: 1X 10⁴ copies/ml

Note: Analysis sensitivity depends on the sample volume, elution volume, nucleic acid extraction methods and other factors. If you use the DNA extraction buffer in the kit, the analysis sensitivity is the same as it declares. However, when the sample volume is dozens or even hundreds of times greater than elution volume by some concentrating method, it can be much higher.

5. Storage

- All reagents should be stored at -20°C. Storage at +4°C is not recommended.
- All reagents can be used until the expiration date indicated on the kit label.
- Repeated thawing and freezing (>3x) should be avoided, as this may reduce the sensitivity of the assay.
- Cool all reagents during the working steps.
- Reaction Mix should be stored in the dark.

6. Additionally Required Materials and Devices

- Biological cabinet
- Real time PCR system
- Desktop microcentrifuge for "ependorf" type tubes (RCF max. 16,000 x g)
- Vortex mixer
- Real time PCR reaction tubes/plates
- Cryo-container
- Pipets (0.5 µl – 1000 µl)
- Sterile filter tips for micro pipets
- Sterile microtubes
- Disposable gloves, powderless
- Biohazard waste container
- Refrigerator and freezer
- Tube racks

7. Warnings and Precaution

- Carefully read this instruction before starting the procedure.
- For in vitro diagnostic use only.
- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
- This assay needs to be run according to Good Laboratory Practice.
- Do not use the kit after its expiration date.
- Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.
- Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
- Prepare quickly the Reaction mix on ice or in the cooling block.
- Set up two separate working areas: 1) Isolation of the RNA/ DNA and 2) Amplification/ detection of amplification products.
- Pipets, vials and other working materials should not circulate among working units.

- Use always sterile pipette tips with filters.
- Wear separate coats and gloves in each area.
- Do not pipette by mouth. Do not eat, drink, smoke in laboratory.
- Avoid aerosols.

8. Sample Collection, Storage and transport

- Collect samples in sterile tubes.
- Specimens can be extracted immediately or frozen at -20°C to -80°C.
- Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents.

9. Procedure

9.1 DNA-Extraction

DNA extraction buffer is supplied in the kit, please thaw the buffer thoroughly and spin down briefly in the centrifuge before use.

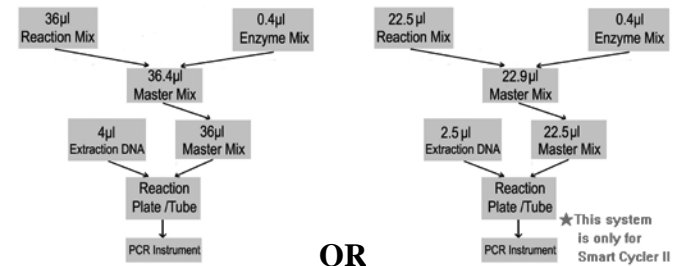
- 1) Pipet 50µl non-heparin anticoagulation sample to a new 0.5ml tube, add 50µl DNA extraction buffer, close the tube then vortex for 10 seconds. Spin down briefly in a table centrifuge.
- 2) Incubate the tube for 10 minutes at 100°C.
- 3) Centrifuge the tube at 13000rpm for 10 minutes. The supernatant contains DNA extracted and can be used for PCR template.

Attention:

- A. During the incubation, make sure the tube is not open. Since the vapor will volatilize into the air and may cause contamination if the sample is positive.
- B. The extraction sample should be used in 3 hours or stored at -20°C for one month.
- C. Different DNA extraction kits are available. You may use your own extraction systems or the commercial kit based on the yield. For the DNA extraction, please comply with the manufacturer's instructions.

9.2 PCR Protocol

The Master Mix volume for each reaction should be pipetted as follows:



OR

- 1) The volumes of Reaction Mix and Enzyme Mix per reaction multiply with the number of samples, which includes the number of controls, standards, and sample prepared. Molecular Grade Water is used as the negative control. For reasons of unprecise pipetting, always add an extra virtual sample. Mix completely then spin down briefly in a centrifuge. Mix completely then spin down briefly in a centrifuge.
- 2) Pipet 36µl (22.5µl for SmartCycler II) Master Mix with micropipets of sterile filter tips to each of the real time PCR reaction plate/tubes. Separately add 4µl (2.5µl for SmartCycler II) DNA sample, positive and negative controls to different reaction plate/tubes. Immediately close the plate/tubes to avoid contamination.
- 3) Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.
- 4) Perform the following protocol in the instrument:

94°C for 2min	1cycle
93°C for 10sec, 62°C for 40sec	30cycles
(Fluorescence measured at 62°C)	

FAM	Mutant type
HEX/VIC/JOE	Wild type

- 5) Δ If you use ABI Prism® system, please choose "none" as passive reference and quencher.

10. Threshold setting:

In channel FAM, just above the maximum level of wild type positive control.
In channel HEX/VIC/JOE, just above the maximum level of mutant type positive control.

11. Quality control: Negative control and positive control must be performed correctly, otherwise the sample results is invalid.

Control	Channel	Ct value	
		Channel FAM	Channel HEX/VIC/JOE
Molecular Grade Water		UNDET	UNDET
Wild type positive control		—	≤25
Mutant type positive control		≤25	—

12. Data Analysis and Interpretation

The following sample results are possible:

	Ct value	Result Analysis
1#	UNDET	Below the detection limit or negative
2#	≤28	Positive; Channel FAM: The sample contains Mitochondrion DNA site G11778A Mutation gene DNA. Channel HEX/VIC/JOE: The sample contains wild type Mitochondrion gene DNA.
3#	28~30	Re-test; If it is still 28~30, report as 1#