Human APOE genotypes (ε2/ε3/ε4) Real Time PCR Kit
User Manual
For Research Use Only

Cat. No.: 0743-ND-01
Lot. No.: (See product label)

1. Introduction

Creative Biogene Human APOE genotypes (ε2/ε3/ε4) Real Time PCR Kit is used for the detection of 6 types ε2/ε2 (rs429358T/T, rs7412T/), ε2/ε3 (rs429358T/T, rs7412T/C), ε3/ε3 (rs429358T/T, rs7412C/C), ε2/ε4 (rs429358T/C, rs7412T/C), ε3/ε4 (rs429358T/C, rs7412C/C), ε4/ε4 (rs429358C/C, rs7412C/C) of APOE gene in whole blood sample by using real time PCR systems. The kit contains a specific ready-to-use system for the detection of the 6 types of APOE gene using PCR (polymerase chain reaction) in the real-time PCR system.

2. Intended Use

Human APOE genotypes (ε2/ε3/ε4) Real Time PCR Kit is used for the detection of 6 types ε2/ε2 (rs429358T/T, rs7412T/), ε2/ε3 (rs429358T/T, rs7412T/C), ε3/ε3 (rs429358T/T, rs7412C/C), ε2/ε4 (rs429358T/C, rs7412T/C), ε3/ε4 (rs429358T/C, rs7412C/C), ε4/ε4 (rs429358C/C, rs7412C/C) of APOE gene in whole blood samples by using real time PCR systems.

3. 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 (Ct) is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities during Real Time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

4. Product Description

Apolipoprotein E (APOE) is a protein involved in the metabolism of fats in the body of mammals. A subtype is implicated in Alzheimer's disease and cardiovascular disease. APOE is polymorphic, with three major alleles (epsilon 2, epsilon 3, and epsilon 4): APOE-ε2 (cys112, cys158), APOE-ε3 (cys112, arg158), and APOE-ε4 (arg112, arg158). Although these allelic forms differ from each other by only one or two amino acids at positions 112 and 158, these differences alter APOE structure and function. Human APOE genotypes (ε2/ε3/ε4) Real Time PCR Kit contains a specific ready-to-use system for the detection of 6 types of APOE gene by polymerase chain reaction in the real-time PCR system. Fluorescence is emitted and measured by the real time systems´ optical unit during PCR.

5. Kit Contents

Ref.	Type of reagent	12rxns	24rxns
1	8-Well PCR Tube Strips	8-Well PCR Tube Strip*6	8-Well PCR Tube Strip*12
2	Positive Control	1 vial, 100μl	1 vial, 200μl
3	Negative Control	1 vial, 100μl	1 vial, 200μl

Analysis sensitivity: 0.2ng/ uL(genome DNA sample)；Ct≤5.0%
Note: Analysis sensitivity depends on the sample volume, elution volume, nucleic acid extraction methods and other factors. If you use the DNA extraction kits recommended, 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.

6. 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.
- Super mix should be stored in the dark.

7. Additionally Required Materials and Devices

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

8. 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.

9. Sample Collection, Storage and transportation

- This kit is suitable for the detection of human genomic DNA extracted from fresh whole blood collected in sodium citrate anticoagulation tubes and EDTA anticoagulation tubes, and blood collected in heparin anticoagulation tubes should be avoided.
- The purity and concentration of the extracted DNA were determined by UV spectrophotometer. The OD260/280 of the sample should be between 1.6 and 2.0, and the DNA concentration should be greater than 10ng/uL
- After the sample is collected, it should be tested as soon as possible. If it cannot be detected in time, it should be stored at -20°C

to 5'°C to avoid repeated freezing and thawing.

•The transportation of whole blood must comply with relevant national and local regulations, and the extracted DNA should be transported in ice packs.

10. Procedure

1) Different brand 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. The recommended Extraction kit is as follows: DNA Isolation Kit (Magnetic Beads Column Method) (Creative Biogene NKR-T001-ND-01).

2) Thaw the required amount of 8-Well PCR Tube Strips, positive control and negative control, label them well, Shake and mix，and centrifuge for 15s for later use.

Note: Each 8-Well PCR Tube Strip detects 2 samples. 8-Well PCR Tube Strips are marked with numbers 1~8.

Recommended setting is as follows:

Number	**1**	2	3	4
Sample	Sample 1			
Detection system	**Tube 1**	Tube 2	Tube 3	Tube 4
Fluorescence signal	VIC, FAM	VIC, FAM	VIC, FAM	VIC, FAM
Number	**5**	6	7	8
Sample	Sample 2			
Detection system	**Tube 1**	Tube 2	Tube 3	Tube 4
Fluorescence signal	VIC, FAM	VIC, FAM	VIC, FAM	VIC, FAM

3) Separately add 5μl DNA sample, positive and negative controls to 8-Well PCR tube strips. Immediately close the plate/tubes to avoid contamination.

4) Put the 8-Well PCR Tube Strips into the real-time PCR instrument and record the sequence of the reaction tubes.

95°C for 2min	1cycle
95°C for 10sec 72°C for 30sec	10cycles
95°C for 10sec, 60°C for 1min 72°C for 30sec (Fluorescence measured at 60°C)	30cycles
Selection of fluorescence channels	
FAM	Internal Control
HEX/VIC/JOE	Target Nucleic Acid

11. Determination of the baseline: In the experiment, the curve with less fluctuation and more stable is generally selected as the baseline, and the user can adjust it according to the actual situation. The end point is to avoid covering where the signal has started to grow significantly. And the principle is that the interval between the starting point and the ending point should preferably be more than 12 cycles.

12. Threshold setting: just above the maximum level of negative control.

13. Quality control:

1) If FAM channel is no amplification curve, it means that the added DNA contains PCR inhibitors, and it is necessary to re-extract the DNA.

2) If the DNA internal control FAM signal of the sample to be tested is normal, proceed to the next step.

3)Negative control: Ct value of FAM channels is ⩽ 23.0, HEX/VIC/JOE channels have no amplification curve, or the amplification curve is linear with no obvious exponential growth phase.

4) Positive control:

	Ct value	
	FAM	HEX/VIC/JOE
Tube 1	⩽23.9	⩽24.9
Tube 2	⩽24.3	⩽25.2
Tube 3	⩽23.7	⩽25.7
Tube 4	⩽24.4	⩽27.4

The internal Control FAM channel Ct value should be less than 25. When the internal Control value is normal, calculate the difference between the target channel Ct (VIC) values of tube 1 and tube 2, tube 3 and tube 4.△Ct1 = Ct (tube1) (VIC) – Ct (tube2) (VIC) or △Ct2 = Ct (tube3) (VIC)- Ct (tube4) (VIC).

	△Ct	Tube			
		1	2	3	4
1	-5⩽△Ct1⩽5	+	+	/	/
2	△Ct1＜-5 or tube2 (VIC) has no Ct value	+	-	/	/
3	△Ct1＞5 or tube1 (VIC) has no Ct value	-	+	/	/
4	-5⩽△Ct2⩽5	/	/	+	+
5	△Ct2＜-5 or tube4 (VIC) has no Ct value	/	/	+	-
6	△Ct2＞5 or tube3 (VIC) has no Ct value	/	/	-	+

14. Data Analysis and Interpretation ： The following sample results are possible:

Negative control and positive control are required for each experiment;

	Tube				Result Analysis		
	1	2	3	4	rs429358	rs7412	genotypes
1	+	-	+	-	TT	TT	ε2/ε2
2	+	-	+	+	TT	TC	ε2/ε3
3	+	-	-	+	TT	CC	ε3/ε3
4	+	+	+	+	TC	TC	ε2/ε4
5	+	+	-	+	TC	CC	ε3/ε4
6	-	+	-	+	CC	CC	ε4/ε4
7	In addition to the above cases, please re-test						

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