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Human APOE Genotyping qPCR Assay Kit (APOE)

Catalog #9068
50 reactions

Product Description

Apolipoprotein E (ApoE) is a key protein involved in lipid transport and cholesterol homeostasis in both the brain and peripheral tissues. Variations in the APOE gene, resulting from two single nucleotide polymorphisms (rs429358 and rs7412), give rise to three major alleles (ε2, ε3, and ε4) that influence susceptibility to cardiovascular and neurodegenerative diseases. ScienCell's Human APOE Genotyping qPCR Assay Kit (APOE) enables direct genotyping from cells without DNA isolation, using a nested PCR strategy followed by allele-specific amplification to determine the genotype. This design ensures high specificity and sensitivity, with positive and negative reactions fully concordant with sequencing results. The assay is robust, reliably genotyping samples with a limited number of cells.

The APOE kit can use either cell lysate or purified genomic DNA as the PCR template. The included cell lysis buffer and enhancer (Cat# GQ400a and GQ400b) are used to lyse pelleted cell samples. MB6038 master mix is provided for the initial PCR reaction, and the 2X GoldNStart TaqGreen qPCR Master Mix (Cat# MB6018a-1), a SYBR® Green–based master mix, is supplied for the qPCR step. The Nested PCR Outer Primer Set amplifies a larger region of the APOE and ACTB genes, while the E2, E3, and E4 primer sets detect the corresponding APOE alleles. The ACTB primer set amplifies the beta actin gene as an internal control. All primer sets have been validated by PCR and gel electrophoresis for amplification specificity. The reference positive control consists of plasmids containing the E2, E3, and E4 APOE alleles along with the ACTB gene, and serves as a positive control for the assay.

Kit Components

Cat #	Component	Quantity	Storage
MB6018-1	GoldNStart TaqGreen qPCR Master Mix	2 vials	-20°C
MB6038	EmeraldNStart HiFi Marathon PCR Master Mix	1 vial	-20°C
GQ400a	Cell Lysis Buffer	2.5 mL	-20°C
GQ400b	Cell Lysis Buffer Enhancer, 100x	50 µL	-20°C
9068a	Nested PCR Outer Primer Set	100 µL	-20°C
9068b	E2 qPCR Primer Set	100 µL	-20°C
9068c	E3 qPCR Primer Set	100 µL	-20°C
9068d	E4 qPCR primer Set	100 µL	-20°C
9068e	ACTB qPCR Primer Set	100 µL	-20°C
9068f	Nuclease-free H_2O	10 mL	-20°C
9068g	Positive control	100 µL	-20°C

Additional Materials Required (Materials Not Included in Kit)

Customers' samples
qPCR plate or tube
PCR Tubes

Quality Control
The primer sets and positive control were validated by qPCR and Sanger sequencing.

Product Use
APOE is for research use only. It is not approved for human or animal use, or for application in clinical or *in vitro* diagnostic procedures.

Shipping and Storage
The product is shipped on dry ice. Upon receipt, store the all the kit components at -20°C. Once thawed, do NOT refreeze GoldNStart TaqGreen qPCR master mix (Cat #MB6018a-1), and keep in the dark at 4°C or on ice at all times.

Procedures

Important: ***Only*** *use polymerases with hot-start capability to prevent possible primer-dimer formation.* ***Only*** *use nuclease-free reagents in PCR amplification.*

Preparation of Lysis Mix

1. Prepare 50 µL of lysis buffer for each sample according to the instructions.
2. Add 1 µL Cell Lysis Buffer Enhancer into 99 µL lysis buffer. Mix gently.

Preparation of cell lysate samples
Note: *If genomic DNA (gDNA) from the samples is already available, skip this step.*

1. Collect 5,000–500,000 cells for each sample, wash with PBS, and pellet.
2. Add 50 µL of lysis buffer to each pellet. Break up the pellet and vortex for 15 seconds.
3. Incubate the samples at 60°C for 7 minutes, followed by 98°C for 3 minutes.
4. Samples can be stored at -20°C for up to 12 months.

B. PCR setup

1. Allow Cat# MB6038, Cat# 9068a and Cat# 9068f vials to warm to room temperature.
2. For each cell lysate or gDNA sample, prepare a PCR reaction. Prepare 20 µl PCR reactions for one well as shown in Table 1.
3. Prepare a "no-lysate" negative control PCR reactions by using 2 µL of supplemented lysis buffer as the PCR template (no cell lysate added). Prepare 20 µl PCR reactions for one well as shown in Table 1.

Table 1. Sample Preparation

Cell lysate or gDNA (1-100 ng) or cell lysis buffer	2 µl
Nested PCR Outer Primer Set (Cat# 9068a)	2 µl
PCR master mix (Cat# MB6038)	10 µl
Nuclease-free H_2O (Cat #9068f)	6 µl
Total volume	***20 µl***

4. Seal the PCR reaction wells. Centrifuge the PCR plates or tubes at 1,500x g for 15 seconds.
5. Run PCR reactions, followed by analyzing the PCR products. For PCR program setup, refer to Table 2.

Table 2. Recommended PCR Program

Step	Temperature	Time	Number of cycles
Pre-incubation	95°C	10 min	1
Denaturation	95°C	30 sec	35
Annealing	62°C	30 sec	
Extension	72°C	30 sec	
Hold	4°C		

C. qPCR setup

1. Prior to use, allow the cat# MB6018-1 and cat# 9068b-9068g to thaw at room temperature. Shake gently to mix well.
2. Take the PCR products and dilute 150X with Nuclease free water. For example, take 1 ul and mix with 149 µl of nuclease free water.
3. For each diluted PCR product, positive control (Cat #9068g) and negative control, prepare 4 qPCR reactions, with E2, E3, E4 and ACTB primers. Prepare 20 µl qPCR reactions for one well as shown in Table 3.

Table 3

Positive control, diluted PCR product	2 µl
GoldNStart TaqGreen qPCR Master Mix (Cat # MB6018-1)	10 µl
qPCR primer set (E2, E3, E4 or ACTB)	2 µl
Nuclease-free H_2O (Cat #9068c)	6 µl
Total volume	***20*** µl

4. Seal the qPCR reaction wells. Centrifuge the plates or tubes at 1,500x g for 15 seconds. For maximum reliability, replicates are highly recommended (minimum of 3).

5. Refer Table 4 for qPCR program setup. The 2X GoldNStart TaqGreen qPCR master mix (Cat. #MB6018a-1) contains SYBR®Green as the reporter dye and does not contain a ROX passive reference dye. If the qPCR instrument being used has a "ROX passive reference dye" option, please deselect this option.

Table 4

Step	Temperature	Time	Number of cycles
Initial denaturation	95°C	10 min	1
Denaturation	95°C	20 sec	28
Annealing/Extension	63°C	50 sec	
Data acquisition	Plate read		

Analysis and Interpretation

Each sample is tested using four primers: E2, E3, E4, and ACTB. ACTB serves as an internal control and must amplify in all samples with Ct < 25 to validate the reaction.

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The APOE genotype is determined by comparing Ct values from the allele-specific reactions (E2, E3, E4):

Ct Comparison	Interpretation
One allele has lowest Ct, and ΔCt with other two ≥5	Sample is homozygous for the allele with lowest Ct.
Two alleles have similarly low Ct, ΔCt <5 between them, and ≥5 vs third allele	Sample is heterozygous for the two low-Ct alleles.
All three alleles show Ct values within <5 cycles of each other, but all are Ct >25.	Ensure the initial PCR reaction was successful by checking it on the gel. Increase the PCR product input in the qPCR reaction.

Steps for Analysis:

1. Confirm ACTB Ct <25. If ACTB fails, the sample is invalid.
2. For E2, E3, E4 reactions Ct>25 is considered negative.
3. Identify the allele(s) with the lowest Ct values.
4. Calculate ΔCt between the lowest Ct allele(s) and the others.
5. Apply the rules in the table to determine genotype:
    - ΔCt ≥5 indicates the higher-Ct allele is negative.
    - ΔCt <5 between two low-Ct alleles indicates the sample is positive for both (heterozygous).

Data Analysis and Interpretation Examples:

	E2	E3	E4	ACTB	Genotype
Ct value	22.81	13.01	13.67	12.5	ε3/ε4 heterozygous
	25	15.4	Not detected	14.3	ε3 homozygous