

## EzWay™ Direct ApoE Genotyping Kit

- 1. Catalog No.** K0568500
- 2. No. of Applications** 50 reactions
- 3. Storage** 1 year at -20°C, 1 month at 4°C  
Avoid repeated freezing and thawing.

**4. Contents**

Component	Cat. No	Volume	Comment
2X EzWay™ Direct Hot Taq PCR MasterMix	K0568210	650ul	Mixture of Direct PCR Buffer, Hot Taq DNA Polymerase, dNTP, MgCl <sub>2</sub> , Red dye and additive
2.5X ApoE PrimerMix	K0568501	500ul	Primer mixture for ApoE gene
Size Marker	K0570111S	50ul	112, 253, 308, 444, 514bp
Positive Control*	-	10ul	Mixture of DNAs (E2/E4)

\* Positive Control provided in this kit is compatible with Direct PCR only. It may cause inhibition at the general PCR condition.

**5. Description**

Human Apolipoprotein E (ApoE) is a 34 kDa glycoprotein that plays a central role in lipid metabolism and transportation. The ApoE gene is polymorphic with three common alleles, designated E2, E3, and E4. These genes encode three ApoE protein isoforms, E2 (Cys112/Cys158), E3 (Cys112/Arg158) and E4 (Arg112/Arg158), that differ by cysteine-arginine interchanges at sites 112 and 158 in the polypeptide chain. The common ApoE isoforms exhibit variations in structure and function and are involved in several pathological processes. For instance, familial type III hyperlipoproteinemia is associated with the ApoE E2/E2 pheno/genotype, while the ApoE E4/E4 pheno/genotype is associated with high cholesterol levels, coronary artery disease, and Alzheimer's disease.

EzWay™ Direct ApoE Genotyping Kit is useful for simple ApoE genotyping from whole blood without DNA extraction by using direct PCR technology. Also, the primer mixture of ApoE genes enables to perform one-step Multiplex PCR.

- ApoE genotyping directly from blood
- One-step Multiplex PCR system with PrimerMix
- ApoE primer mixture for E2 (Cys112/Cys158), E3 (Cys112/Arg158) and E4 (Arg112/Arg158)
- Fast and simple method for genotyping without DNA purification
- Minimize the risk of DNA loss or contamination
- Optimized MasterMix type containing Hot Taq enzyme, dNTP, Direct PCR Buffer, MgCl<sub>2</sub>, Red dye and additives
- Useful detection of high cholesterol levels, cardiovascular disease or Alzheimer's disease

**6. Procedure**

This kit does not require DNA purification from whole blood. Thus you perform gene amplification from blood directly. All operations are performed on ice or in the cooling box. If you use anticoagulant treated container to obtain blood, you must fill blood by the amount recommended by the manufacturer.

1. Determine the total number of samples including negative control and prepare PCR tubes.
2. Thaw 2X EzWay™ Direct Hot Taq PCR MasterMix and 2.5X ApoE PrimerMix, and vortex.

**Note:** Mix the reagents completely.

3. Spin the tubes briefly in a microcentrifuge.
4. Dispense 12.5ul 2X EzWay™ Direct Hot Taq PCR MasterMix, 10ul ApoE PrimerMix, 1.5ul distilled water and 1ul blood sample or a positive control into PCR tubes, and then mix thoroughly. **At this time, do not use blood sample after spin down.** For no template control (NTC), put 1ul distilled water into a PCR tube instead of blood.
5. Place the PCR tubes in a thermal cycler and perform the PCR reaction immediately.

Step		Temp.	Time	Cycles
<b>Initial Denaturation</b>		95°C	15 min	1
<b>Cycling</b>	Denaturation	95°C	0.5 min	35
	Annealing	65°C	0.5 min	
	Extension	72°C	1 min	
<b>Final Extension</b>		72°C	10 min	1

6. Separate PCR products and size marker on 2% agarose gel electrophoresis in 1X or 0.5X TAE buffer and stain with EtBr or GelRed (Biotium, Inc.).

**Note:** Do not use TBE buffer because of band smearing and poor resolution.

**7. Result**

**Table 1. Amplicons by APOE polymorphisms**

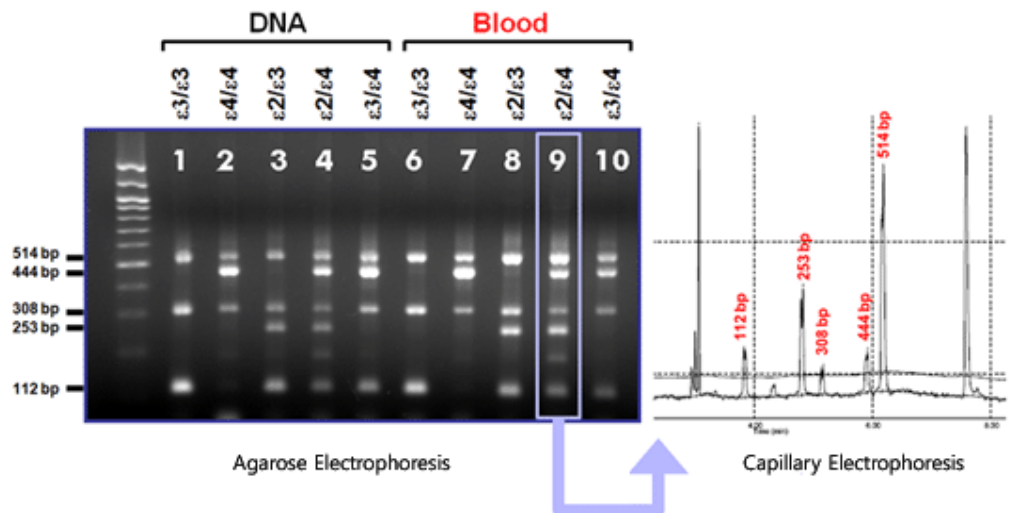
Genotype	1 <sup>st</sup> , codon 112 <sup>a</sup>		2 <sup>nd</sup> , codon 158 <sup>a</sup>		Common Amplicon (bp)
	Nt / AA <sup>b</sup>	Amplicon (bp)	Nt / AA <sup>b</sup>	Amplicon (bp)	
E2/E2	TGC / Cys	112	TGC / Cys	253	514
E3/E3	TGC / Cys	113	CGC / Arg	308	
E4/E4	CGC / Arg	444	CGC / Arg	308	
E2/E3	TGC / Cys	113	TGC / Cys CGC / Arg	253 308	
E2/E4	TGC / Cys CGC / Arg	113 444	TGC / Cys CGC / Arg	253 308	
E3/E4	TGC / Cys CGC / Ar	113 444	CGC / Arg	308	

a. The mark of 1st and 2nd is order of mutation sequence in GenBank, and codon number is corresponded to each mutation.

b. Nt / AA is nucleotide / amino acid at mutation site.

**Direct ApoE genotyping from Blood**

Blood was amplified directly using EzWay™ Direct ApoE Genotyping Kit. One-step Multiplex PCR enables to get the result just in 3 hours.



**8. Trouble Shooting**

<b>Problem</b>	<b>Probable Cause</b>	<b>Solution</b>
No band	Pipetting error	Check pipettes.
	Problem with thermal cyclers or PCR cycling conditions	Check your thermal cycler and PCR condition.
	Reagents and Blood	Check the storage conditions and the expired date of kit reagents. Check your blood samples. If blood samples are obtained with anticoagulant treated container, you must identify whether you have drained blood by the recommended amount out of the container's manufacturer. Check the dispensed amount of blood because of high viscosity of blood.
	Agarose buffer	Use TAE buffer to avoid poor resolution or band smearing.
Faint band	Pipetting error	Check pipettes.
	Problem Reagents	Check the storage conditions and the expired date of kit reagents.
Smear band	Carry-over contamination	PCR reagents or consumables may be contaminated.
	Agarose buffer	Use TAE buffer to avoid poor resolution or band smearing.