# 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 freeze and thaw.

**4. Contents**

| Component | Cat. No | Volume | Comment |
|---|---|---|---|
| 2X EzWay™ Direct Taq PCR MasterMix | K0568010 | 625ul | Mixture of Direct PCR Buffer, Taq DNA Polymerase, dNTP, $MgCl_2$, Red dye and additive |
| 2.5X ApoE PrimerMix | K0568501 | 500ul | Primer mixture for ApoE gene |
| Distilled Water | K0568502 | 500ul | PCR Grade |
| Size marker | K0570111S | 50ul | 100bp Ladder |

**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 ε2, ε3, and ε4. 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 contains Direct Taq PCR MasterMix, a new cocktail solution that facilitates polymerase chain reaction (PCR) without DNA isolation. Direct PCR Buffer in this MasterMix neutralizes inhibitors present in blood and allows direct ApoE genotyping from blood without any DNA extraction. 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 Taq enzyme, dNTP, Direct PCR Buffer, $MgCl_2$, Red dye and additives
- Useful detection of high cholesterol levels, cardiovascular disease or Alzheimer's disease



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## 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 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 Taq PCR MasterMix, 10ul ApoE PrimerMix, 1.5ul distilled water and 1ul blood sample into PCR tubes, and then mix thoroughly. For negative control, 1ul distilled water instead of blood is put into PCR tube.

5. Place the PCR tubes in a thermal cycler and perform the PCR reaction immediately.

| Step | | Temp. | Time | Cycles |
|---|---|---|---|---|
| **Initial Denaturation** | | 95°C | 15 min | 1 |
| **Cycling** | Denaturation | 94°C | 0.5 min | 40 |
| | Annealing | 68°C | 0.5 min | |
| | Extension | 72°C | 1 min | |
| **Final Extension** | | 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.).

   The amplified DNA can be detected by various electrophoresis techniques. The most common techniques are agarose or polyacrylamide gel electrophoresis depending on the size of the amplicon.
   **TAE gel is highly recommended** because the bands may be detected dispersed and/or distorted in TBE gel.



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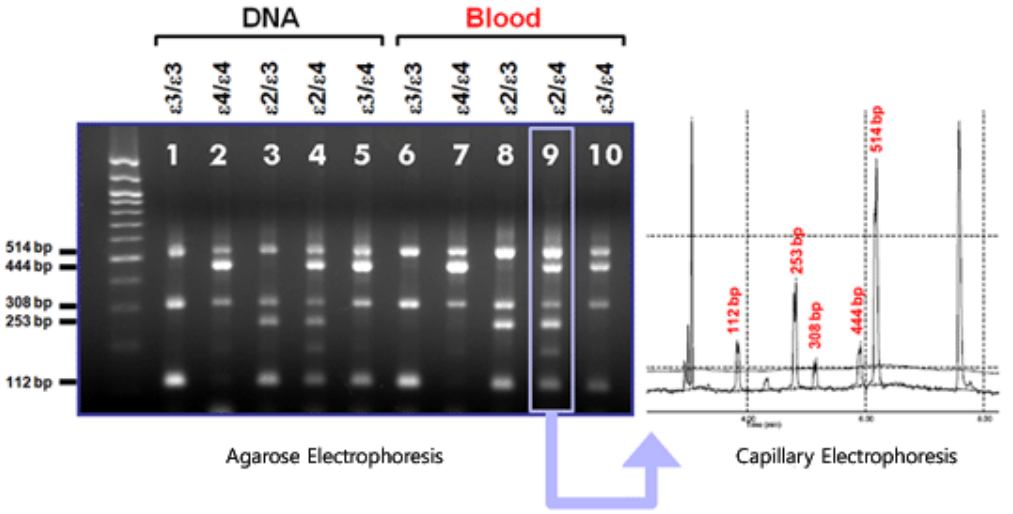
## 7. Result

**Table 1. Amplicons by APOE polymorphisms**

| Genotype | 1st, codon 112 [a] | | 2nd, codon 158 [a] | | Common Amplicon (bp) |
|---|---|---|---|---|---|
| | Nt / AA [b] | Amplicon (bp) | Nt / AA [b] | Amplicon (bp) | |
| ε2/ε2 | **T**GC / Cys | **113** | **T**GC / Cys | **253** | **514** |
| ε3/ε3 | **T**GC / Cys | **113** | **C**GC / Arg | **308** | |
| ε4/ε4 | **C**GC / Arg | **444** | **C**GC / Arg | **308** | |
| ε2/ε3 | **T**GC / Cys | **113** | **T**GC / Cys<br>**C**GC / Arg | **253**<br>**308** | |
| ε2/ε4 | **T**GC / Cys<br>**C**GC / Arg | **113**<br>**444** | **T**GC / Cys<br>**C**GC / Arg | **253**<br>**308** | |
| ε3/ε4 | **T**GC / Cys<br>**C**GC / Arg | **113**<br>**444** | **C**GC / 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.



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## 8. Trouble Shooting

| Problem | Probable Cause | Solution |
|---|---|---|
| No band | Pipetting error | Check pipettes. |
| | Problem with thermal cycler 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.<br>Check your blood samples. If blood samples are obtained with anticoagulant treated container, you must identify whether you have drained blood the amount recommended by the container's manufacturer.<br>Check the dispensed amount of blood because of high viscosity of blood. |
| 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 | We recommended to use TAE buffer not TBE buffer, and high resolution agarose. |



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