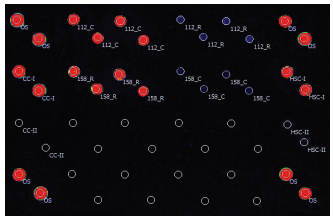
EUROArray APOE Direct



- **Determination of the APOE gene variants ε2, ε3 and ε4 in one test**
- **Highly reliable results owing to numerous intergrated controls**
- **Direct use of EDTA blood: no separate DNA isolation required**

Technical data

Substrate	Single-stranded DNA probes, length: 20 to 50 nucleotides
Test procedure	DNA extraction / PCR (approx. 60 min) / hybridisation (60 min) / fully automated evaluation; total working time approx. 2 min per sample incl. DNA extraction with the direct method (with 40 samples per run)
Reagents	Ready for use
Controls	DNA-negative control and other integrated controls
CE IVD label	Complete process incl. DNA extraction is validated
Test kit format	5, 10 or 20 slides, each containing 5 test fields, or 8 slides each containing 3 test fields
Order no.	**MN 5710-0505-V, -1005-V, -2005-V, -0803-V**

Clinical significance

The EUROArray APOE Direct is designed for the molecular genetic determination of the APOE alleles ε2, ε3 and ε4, especially within the framework of differential diagnosis and/or early identification of late-onset sporadic Alzheimer's disease and type III hyperlipoproteinemia. Moreover, arteriosclerosis and other vascular diseases (coronary heart disease, stroke) are associated with the presence of particular APOE alleles. Apolipoprotein E (ApoE) also plays an important role in lipometabolism, coagulation, the immune defense, and the protection from oxidation processes. ApoE binds to the amyloid-β peptid, which plays a central role in neurodegeneration in Alzheimer patients. The three different APOE alleles ε2, ε3 and ε4 result in three different isoforms of the ApoE protein (E2, E3 and E4), whose amino acids differ at positions 112 and 158 as described below: E2: cysteine - cysteine; E3: cysteine - arginine; E4: arginine - arginine.

The ε4 allele occurs in Alzheimer patients approximately 3 times as often as in the normal population (36.7 % vs. 13.7 %), whereas the ε2 allele occurs more seldom than in the normal population (3.9 % vs. 8.4 %). Correspondingly, carriers of the APOE-ε4 allele have a higher risk of developing Alzheimer's disease, while the ε2 allele is associated to a lower risk.

Relative risk of developing Alzheimer's disease in comparison to ε3/ε3 carriers:

ε2/ε2	ε2/ε3	ε2/ε4	ε3/ε3	ε3/ε4	ε4/ε4
<0.1fold	<0.1fold	<2fold	<1fold	<3fold	<5–8fold

In families with a history of late-onset sporadic Alzheimer's disease, the risk of developing the disease and the average start of the disease depend strongly on the ε4 gene dose. 20 % and 84 years for non-ε4-carriers, 47 % and 76 years for heterozygous and 91 % and 68 years for homozygous carriers of the ε4 allele. Alongside its diagnostic significance, the determination of the APOE alleles is gaining more and more pharmacogenetic importance in the development of new Alzheimer medications.

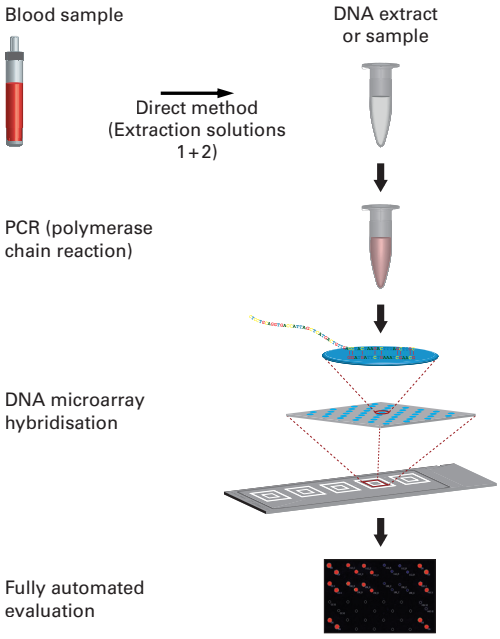
Diagnostic application

The EUROArray APOE Direct allows fast and simple determination of the APOE gene variants ε2, ε3 and ε4 in a single test. The direct method enables the direct use of whole blood samples and eliminates the need for time- and cost-intensive DNA isolation.

EUROIMMUN AG · Seekamp 31 · 23560 Luebeck (Germany) · Tel +49 451 5855 0 · Fax +49 451 5855 591 · euroimmun@euroimmun.de · www.euroimmun.com

Test principle

EDTA blood (direct method) or isolated genomic DNA from the patient are used as sample material. In the direct method genomic DNA from blood cells is prepared for polymerase chain reaction (PCR) by diluting the blood with the extraction solution provided in the test kit and incubating it for one minute. In the first reaction step, two sections of the APOE gene are amplified by PCR from the extract or, alternatively, from a genomic patient DNA sample. During their formation, both PCR products are labelled with a fluorescent dye. In the second reaction step, the PCR products are analysed using the microarray, which contains immobilised probes that are complementary to the amplified DNA. The specific binding (hybridisation) of the fluorescing PCR product to the corresponding microarray spot is detected using the EUROIMMUN Microarray Scanner. All spot signals are evaluated automatically using the EUROArrayScan software. For each parameter the genotype is deduced from the proportion of signals generated at the allele-specific probes.



Test procedure

For direct use of EDTA blood, the sample is first incubated with extraction solution 1 for one minute and then extraction solution 2 is added. For PCR an aliquot of the extract or alternatively a purified DNA sample is mixed with the ready-made PCR reagents. The PCRs are incubated in the thermocycler and then, using the TITERPLANE technique, on EUROArray slides containing microarray BIOCHIPs. Scanning and evaluation are performed using the EUROArrayScan system (Microarray Scanner incl. EUROArrayScan software). This provides fully automated evaluation of EUROArray analyses and detailed documentation of results.

Sensitivity and specificity

Specificity and sensitivity of the test system were determined with samples precharacterised using a molecular genetic method.

Reference samples	Reference method	Sensitivity with respect to the reference method	Specificity with respect to the reference method
71 EDTA blood samples from blood donors, Germany	molecular genetic	100 %	100 %
74 DNA samples from blood donors, Germany	molecular genetic	100 %	100 %

Robustness

For 304 tested DNA samples, the determination was successful in all cases (100 %). For 301 analysed EDTA blood samples the determinations were also successful in all cases (100 %) using the direct method.

Literature

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