Version 22-04/24

User's Manual

Human pan-ApoE ELISA Kit

REF **DEIANS036**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

FOR RESEARCH USE ONLY. Not for clinical diagnosis use. The ELISA Kit provides a quantitative assay for human ApoE2, ApoE3, and ApoE4 proteins.

General Description

Apolipoproteins are plasma lipoproteins that function as transporters of lipids and cholesterol in the circulatory system. Chylomicrons are a fundamental class of apolipoproteins containing very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL). Human ApoE has three isoforms: ApoE2, ApoE3, and ApoE4. These three isoforms differ in the combination of cysteine and arginine residues located at positions 130 and 176. The ApoE4 isoform contains arginine at both locations. Research studies have linked ApoE4 function to neuronal plasticity, synaptogenesis, and neurodegenerative diseases. ApoE4 is produced in the liver and brain, although it is widely expressed in other tissues, such as the lung, spleen, and ovary. Investigators have established the ApoE4 allele as a genetic risk factor for Alzheimer's disease (AD), accounting for 50-60% of the genetic variation in the disease. Research studies indicate that patients expressing ApoE4 have a reduced capacity for synaptic plasticity, an earlier age of onset of AD, and an increase in amyloid-beta (Aβ) deposition. The increase in Aβ suggests a role for ApoE4 in the impairment of amyloid clearance.

Principles of Testing

The Human pan-ApoE ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of ApoE (ApoE2, ApoE3, and ApoE4). Incubation of cell lysates and detection antibody on the coated microwell plate forms a sandwich with ApoE in a single step. The plate is then extensively washed and TMB reagent is added for signal development. The magnitude of absorbance for the developed color is proportional to the quantity of ApoE.

Reagents And Materials Provided

1. ApoE (pan) Rabbit mAb Coated Microwells, 96 tests, 4°C
2. ApoE (pan) Mouse Detection mAb, 1 each, Lyophilized, 4°C
3. HRP Diluent, 5.5 mL, 4°C
4. TMB Substrate, 11 mL, 4°C
5. STOP Solution, 11 mL, 4°C
6. Sealing Tape, 2 each, 4°C
7. ELISA Wash Buffer (20×), 25 mL, 4°C
8. Cell Lysis Buffer (10×), 15 mL, -20°C

Storage

Kit should be stored at 4°C with the exception of 10× Cell Lysis Buffer, which is stored at -20°C (packaged separately).

Specimen Collection And Preparation

1. For adherent cells

a. Aspirate media when the culture reaches 80–90% confluence. Treat cells by adding fresh media containing regulator for desired time.

b. Remove media and rinse cells once with ice-cold 1× PBS.

c. Remove PBS and add 0.5 mL ice-cold 1× Cell Lysis Buffer including 1 mM PMSF and Protease/Phosphatase Inhibitor Cocktail to each plate (10 cm diameter) and incubate the plate on ice for 5 min.

d. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.

e. Sonicate lysates on ice.

f. Microcentrifuge for 10 min (14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at -80°C in single-use aliquots.

2. For suspension cells

a. Remove media by low speed centrifugation (~1200 rpm) when the culture reaches 0.5–1.0×10^6 viable cells/mL. Treat cells by adding fresh media containing regulator for desired time.

b. Collect cells by low speed centrifugation (~1200 rpm) and wash once with 5-10 mL ice-cold 1X PBS.

c.Cells harvested from 50 mL of growth media can be lysed in 2.0 mL of 1X Cell Lysis Buffer including 1 mM PMSF and Protease/Phosphatase Inhibitor Cocktail.

d. Sonicate lysates on ice.

e. Microcentrifuge for 10 min (14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at -80°C in single-use aliquots.

Reagent Preparation

NOTE: Prepare solutions with deionized/purified water or equivalent.

1. Microwell strips: Bring all to room temperature before opening bag/use. Unused microwell strips should be returned to the original re-sealable bag containing the desiccant pack and stored at 4°C.

2. Detection Antibody: Reconstitute lyophilized Detection Antibody (red colored cake) with 5.5 mL HRP Diluent. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. For best results, use immediately following antibody reconstitution. Unused reconstituted Detection Antibody may be stored for up to 4 weeks at 4°C, although there may be some loss of signal compared to freshly reconstituted antibody.

3. HRP Diluent: Red colored diluent for reconstitution and dilution of the Detection Antibody that is linked to HRP.

4. 1× ELISA Wash Buffer: Prepare by diluting ELISA Wash Buffer (20×) (included in each kit) to 1× with deionized water.

5. 1× Cell Lysis Buffer: Prepare by diluting 10× Cell Lysis Buffer to 1× with deionized water. This buffer can be stored at 4°C for short-term use (1–2 weeks). Recommended: When using to prepare cell lysates, add Protease/Phosphatase Inhibitor Cocktail (not supplied) and 1 mM phenylmethyl-sulfonyl fluoride (not supplied) immediately before use.

6. TMB Substrate: Bring to room temperature before use.

7. STOP Solution: Bring to room temperature before use.

Assay Procedure

Note:

1. This protocol is for this kit that use an HRP directly conjugated to the detection antibody (1-step method).
2. Equilibrate all materials and prepared reagents to room temperature prior to running the assay.

Procedure:

1. Samples should be undiluted or diluted with 1× Cell Lysis Buffer to a 2× protein concentration in order to achieve a final 1× protein concentration upon addition of the Detection Antibody. Individual datasheets for each kit provide a sensitivity curve that serves as a reference for selection of an appropriate starting lysate concentration. The sensitivity curve shows typical results across a range of lysate concentration points.
2. Add 50 µL of each sample to the appropriate wells.
3. Add 50 µL of the Detection Antibody to each well.
4. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm (moderate agitation).
5. Gently remove the tape and wash wells:

   a. Discard plate contents into a receptacle.

   b. Wash 4 times with 1X Wash Buffer, 200 µL each time for each well.

   c. For each wash, strike plates on fresh towels hard enough to remove the residual solution in each well, but do not allow wells to completely dry at any time.

   d. Clean the underside of all wells with a lint-free tissue.
6. Add 100 µL of TMB Substrate to each well. Seal with tape and incubate the plate in the dark for 15 min at room temperature on a plate shaker (400 rpm, moderate agitation) or alternatively for 10 min at 37°C without shaking.
7. Add 100 µL of STOP Solution to each well. Shake gently for a few seconds.

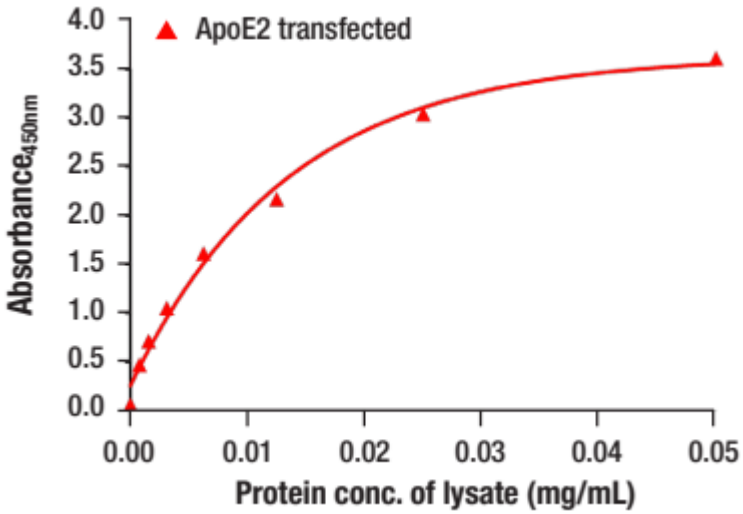
NOTE: Initial color of positive reaction is blue, which changes to yellow upon addition of STOP Solution.

8. Read results:

   a. Visual Determination: Read within 30 min after adding STOP Solution.

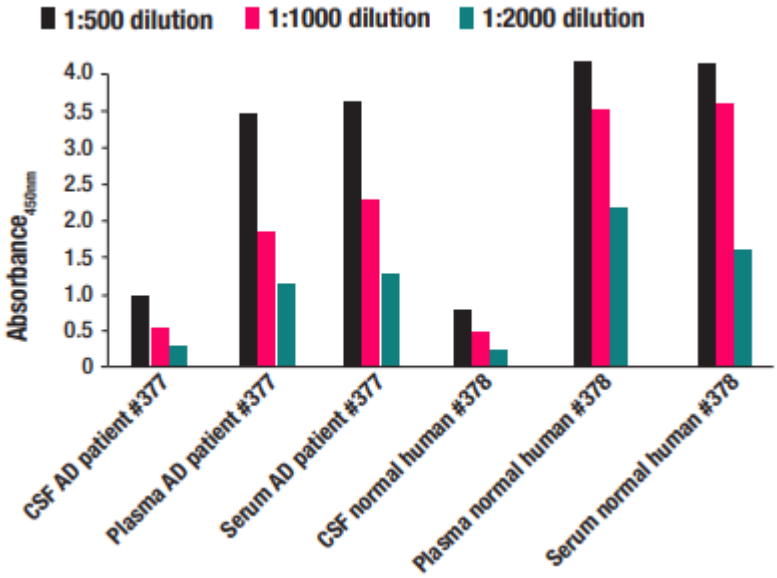
   b. Spectrophotometric Determination: Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP Solution.

Typical Standard Curve



The relationship between lysate protein concentration from 293T cells transfected with a construct expressing full-length human ApoE2 and the absorbance at 450 nm using the Human pan-ApoE ELISA Kit is shown.

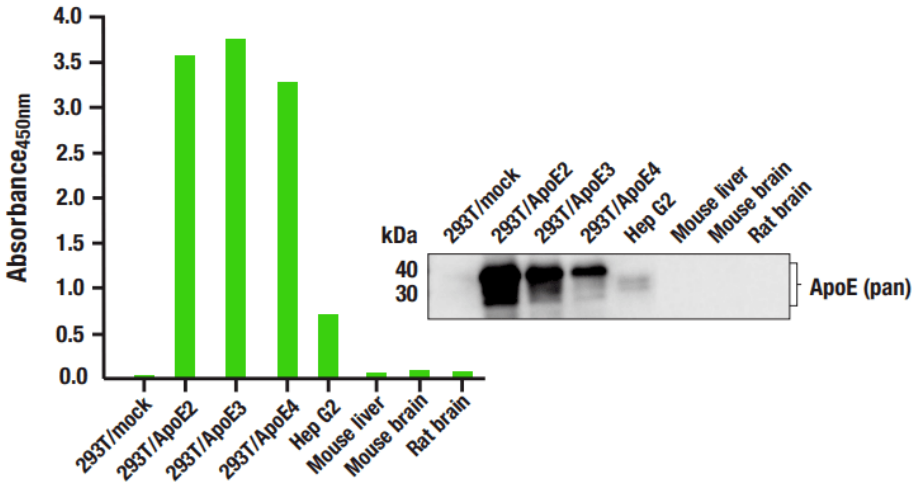
Performance Characteristics



The Human pan-ApoE ELISA Kit detects ApoE protein in human cerebrospinal fluid (CSF), plasma, and serum. The absorbance at 450 nm for 3 dilutions of human CSF, plasma, and serum obtained from one Alzheimer's disease (AD) patient and one normal donor is shown in the figure.

Specificity

The ELISA Kit detects human ApoE2, ApoE3, and ApoE4 proteins.



The Human pan-ApoE ELISA Kit detects human ApoE2, ApoE3, and ApoE4 proteins. The absorbance at 450 nm for a panel of cell and tissue extracts (0.05 mg/mL for transfected cell extracts, 0.2 mg/mL for all other extracts) using the Human pan-ApoE ELISA Kit is shown in the left figure. The corresponding western blot using ApoE (pan) antibody is shown in the right figure (this antibody is human reactive, and not mouse/rat reactive). Extracts were prepared from various cells/tissues, including 293T cells that were either mock transfected (293T/mock), or transfected with constructs expressing full-length human ApoE2 (293T/ApoE2), full-length human ApoE3 (293T/ApoE3), or full-length human ApoE4 (293T/ApoE4).

Precautions

For Research Use Only. Not For Use In Diagnostic Procedures.