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Apolipoprotein E4 (human) ELISA Kit

rev 01/21

(Catalog # K4699-100, 100 assays; Store at -20°C)

I. Introduction:

BioVision's Human Apolipoprotein E4 (ApoE4) Enzyme-Linked Immunosorbent Assay (ELISA) Kit is an in vitro assay for quantitative measurement of human ApoE4. ApoE transports lipoproteins, fat-soluble vitamins, and cholesterol via the lymph system to the blood. ApoE exists as three major isoforms, including ApoE2, ApoE3, and ApoE4. Recently, ApoE4 has been implicated in Alzheimer's disease (AD). ApoE4 is the first risk gene identified in Alzheimer's research, and remains the gene with strongest impact. Everyone inherits a copy of ApoE gene from each parent. Those who inherit one copy of ApoE4 have an increased risk of developing AD (about a quarter of the human population). Those who inherit two copies of ApoE4 have an even higher risk (about 2% of humans with up to 10 times higher risk). In addition to raising risk, ApoE4 may tend to make AD symptoms appear at a younger age. The assay employs an antibody specific for human ApoE4 coated on a 96-well plate. Standard and samples are pipetted into wells and ApoE4 present in the sample is bound to the wells by immobilized antibody. Wells are washed and human ApoE4 specific detection antibody is added. After washing away unbound detection antibody, HRP-conjugate is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells, and color develops in proportion to the amount of bound ApoE4. Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm. Sensitivity of the kit is 25 ng/ml and detection range is from 50 ng to 800 ng/ml. Recovery inside this range is between 83 and 104% (average recovery is 93%). The intra-assay reproducibility as measured by the coefficient of variation (CV) is < 8 % & inter-assay has CV < 12 %.

II. Application:

Quantitative measurement of human Apolipoprotein E4 (ApoE4)

III. Specificity:

Human ApoE4

IV. Sample Type:

- Serum and plasma
- Cell lysate and cell culture supernatant
- Cerebrospinal Fluid (CSF)

V. Kit Contents:

Components	K4699-100	Cap Code	Part No.	Storage Temp.
Plate Coated with ApoE Ab	12 stripsx8 wells	-	K4699-100-1	-20°C
Assay Diluent	100 ml	WM	K4699-100-2	4°C
Wash Buffer A (10x)	10 ml	NM	K4699-100-3	4°C
Wash Buffer B (10x)	20 ml	NM/Brown	K4699-100-4	4°C
rh ApoE4 Standard (100 µg/ml)	3x8 µl	Yellow	K4699-100-5	-20°C
Detection Antibody (100x)	110 µl	Blue	K4699-100-6	-20°C
HRP Conjugate (100x)	135 µl	Green	K4699-100-7	-20°C
TMB Substrate	11 ml	Amber	K4699-100-8	4°C
Stop Solution	11 ml	NM/Blue	K4699-100-9	4°C
Plate Sealer	2	-	K4699-100-10	RT/4°C

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Distilled or deionized water.

VII. Storage Conditions and Reagent Preparation:

Kit can be used within one year if stored unopened at -20°C. Avoid repeated freeze-thaw cycles. After opening the kit, store components according to the Storage temperature listed above. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay. Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

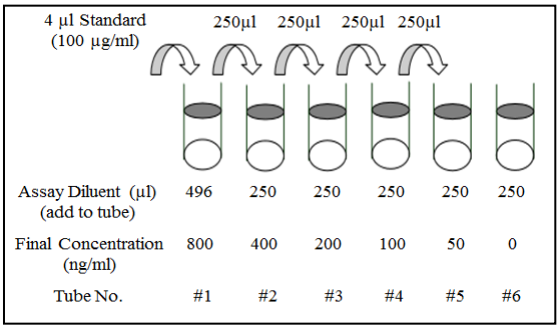
- **Wash Buffer A (10x) and Wash Buffer B (10x):** Dilute with deionized or distilled water to a final working 1x buffer concentration. If the Wash Buffers (10x) contain visible crystals, warm to room temperature and mix gently until dissolved before dilution.
- **rh ApoE4 Standard:** After receiving, store stock Standard solution at -20°C. Avoid freeze/thaw cycle.
- **Detection Antibody**: Dilute 100 fold with Assay Diluent as per assay requirement. Use within couple of hrs.
- **HRP Conjugate**: Dilute only the necessary amount of HRP Conjugate 100 fold in Assay Diluent.

VIII. Assay Protocol:

1. Bring all Buffers and desired number of Ab coated strips to room temperature (18 - 25°C) before use. It is recommended to run all Standard dilutions in duplicate.
2. Dilute sample if required in Assay Diluent. Suggested dilution for normal plasma: 400 fold*.

   * Level of target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.

3. Add 4 µl of stock Standard solution (100 µg/ml) to 496 µl of Assay Diluent to prepare 800 ng/ml Standard solution. Mix thoroughly by pipetting (Do not vortex). Prepare a series of dilution for the 800 ng/ml Standard solution in Assay Diluent as shown in figure. Mix each tube thoroughly before the next transfer. Assay Diluent serves as the zero Standard (0 ng/ml). **Note:** Discard unused Standard solution.



4. Add 100 µl of each Standard and sample into appropriate wells. Cover wells with Plate Sealer and incubate for 1-1.5 hrs at 37°C. Discard the solution and wash 3 times (each wash for 3 min.) with gentle shaking using 200 µl of 1x Wash Buffer A. After last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µl of 1x Detection Antibody solution/well. Incubate for 1 hr at 37°C. Discard the solution. Wash 3 times with 1x Wash Buffer B with gentle shaking.
6. Add 100 µl of 1x HRP Conjugate solution/well. Incubate for 45 min. to 1 hr at 37°C. Discard the solution. Wash 4 times with 1x Wash Buffer B with gentle shaking.
7. Add 100 µl of TMB Substrate/well and gently shake. Measure absorbance at 650 nm for 2-10 min. at room temperature to monitor the blue color development, intensity of which correlates with ApoE4 amount in samples and Standards.

   Notes:

   a. Incubation time after addition of TMB substrate can be optimized to avoid over development of color. Recommended absorbance is ~0.4-0.7 at 650 nm.

   b. Optional: Prepare one parallel well for background control and add TMB Substrate.
8. Add 100 µl of Stop Solution into each well including background control and mix with gentle shaking. Remove air bubbles if any. Read at 450 nm within 5 min.
9. **Calculation:** Calculate the mean absorbance for each set of duplicate Standards. Plot ApoE4 Standard Curve. Calculate ApoE4 concentration of sample by interpolation of the Standard Curve. If sample was diluted, multiply the value by dilution factor to calculate the concentration of human ApoE4 in the sample.

   Note: Background subtraction for each reading is optional for calculating the sample ApoE4 concentration, and will not change the final results.

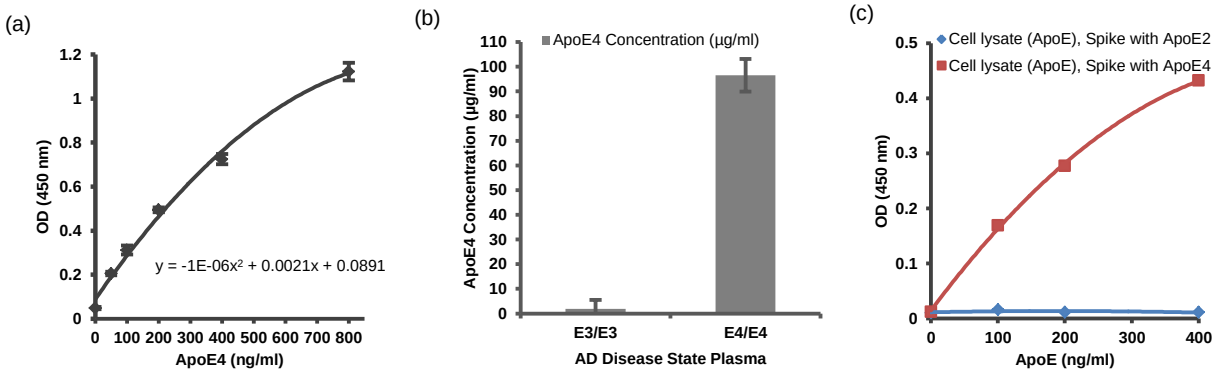


Figure: a) rh ApoE4 Standard Curve. This standard curve is for demonstration only. A standard curve must be run with each assay. b) Measurement of ApoE4 concentration in human plasma samples from Alzheimer's disease (AD) patients following the kit protocol. E3/E3 (left), two copies of ApoE3 in AD patient plasma; E4/E4 (right), two copies of ApoE4 in AD patient plasma. C) Detection of ApoE4 in ApoE overexpressed HEK293T cell line. Cell lysate was spiked with rh ApoE2 or rh ApoE4 and analyzed following the kit protocol.

IX. RELATED PRODUCTS:

ApoE2, human recombinant (4760)
ApoE3, human recombinant (4696)
ApoE4, human recombinant (4699)
ApoE Antibody (CT) (6720)
ApoE2 Antibody (5760)
Apolipoprotein E (human) ELISA Kit (K4696-100)

FOR RESEARCH USE ONLY! Not to be used on humans.