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Apolipoprotein E (human) ELISA Kit

rev 06/17

(Catalog # K4696-100, 100 assays; Store at -20°C)

I. Introduction:

BioVision's Human Apolipoprotein E (ApoE) Enzyme-Linked Immunosorbent Assay (ELISA) Kit is an in vitro assay for the quantitative measurement of human ApoE. ApoE transports lipoproteins, fat-soluble vitamins, and cholesterol via the lymph system to the blood. It is synthesized mainly in liver, while it has also been identified in the brain, kidneys, and spleen. In the nervous system, ApoE is synthesized in non-neuronal cell types, most notably astroglia and microglia, while neurons preferentially express its receptors. Defects in ApoE lead to familial dysbetalipoproteinemia (increased plasma cholesterol and triglycerides). More recently, ApoE has been implicated in several biological processes not directly related to lipoprotein transport, including Alzheimer's disease, immunoregulation, and cognition. The assay employs an antibody specific for human ApoE coated on a 96-well plate. Standard and samples are pipetted into wells and ApoE present in the sample is bound to the wells by immobilized antibody. Wells are washed and human ApoE 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 ApoE. Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm. This ELISA kit recognizes ApoE-2, ApoE-3, and ApoE-4 isoforms. Sensitivity of the kit is 25 ng/ml and detection range is from 25 ng to 1.6 µg/ml. Recovery inside this range is between 86 and 111% (average recovery is 102%). 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 E (ApoE)

III. Specificity:

Human ApoE

IV. Sample Type:

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

V. Kit Contents:

Components	K4696-100	Cap Code	Part No.	Storage Temp.
Plate Coated with ApoE Ab	12 stripsx8 wells	-	K4696-100-1	-20°C
Assay Diluent	100 ml	WM	K4696-100-2	4°C
Wash Buffer A (10x)	10 ml	NM	K4696-100-3	4°C
Wash Buffer B (10x)	20 ml	NM/Brown	K4696-100-4	4°C
rh ApoE Standard	3xLyohilized	Yellow	K4696-100-5	-20°C/-80°C
Detection Antibody (100x)	110 µl	Blue	K4696-100-6	-20°C
HRP Conjugate (100x)	135 µl	Green	K4696-100-7	-20°C
TMB Substrate	11 ml	Amber	K4696-100-8	4°C
Stop Solution	11 ml	NM/Blue	K4696-100-9	4°C
Plate Sealer	2	-	K4696-100-10	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 temp. 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.

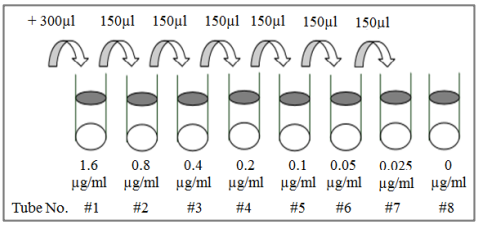
- **Wash Buffer A (10x) and Wash Buffer B (10x):** Dilute with deionized or distilled water to a final working 1x buffer concentration. If the Wash Buffers (10x) contain visible crystals, warm to room temperature and mix gently until dissolved before dilution.
- **rh ApoE Standard:** Add 300 µl Assay Diluent into each Standard vial to prepare 1.6 µg/ml stock Standard solution. Dissolve the powder thoroughly by pipetting. Standard should be stored at -20°C or -80°C (recommended at -80°C) after reconstitution. Reconstitute as per the assay requirement.
- **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 serum: 125-250 fold*.

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

3. Pipette 150 µl Assay Diluent into seven separate tubes and prepare the dilution series of stock Standard solution (1.6 µg/ml) in Assay Diluent as shown in figure. Mix each tube thoroughly before the next transfer. Assay Diluent serves as the zero Standard (0 µg/ml).



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 Solution 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 Solution B.
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 Solution B.
7. Add 100 µl of TMB Substrate/well and gently shake. Measure absorbance at 650 nm for 2-5 min. at room temperature to monitor the blue color development, intensity of which correlates with ApoE amount in samples and Standards.

   Notes:

   a. Incubation time after addition of TMB substrate must be optimized to avoid over development of color. Recommended absorbance is ~0.6-1 at 650 nm.

   b. 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 all bubbles. Read at 450 nm within 5 min.
9. **Calculation:** Calculate the mean absorbance for each set of duplicate Standards, and subtract the reading of the background control from Standard and sample reading. Plot log ApoE Standard Curve. Apply corrected sample reading to the Standard Curve to get ApoE amount in the sample well. If sample was diluted, multiply the value by dilution factor to calculate the concentration of human Apo E in the sample.

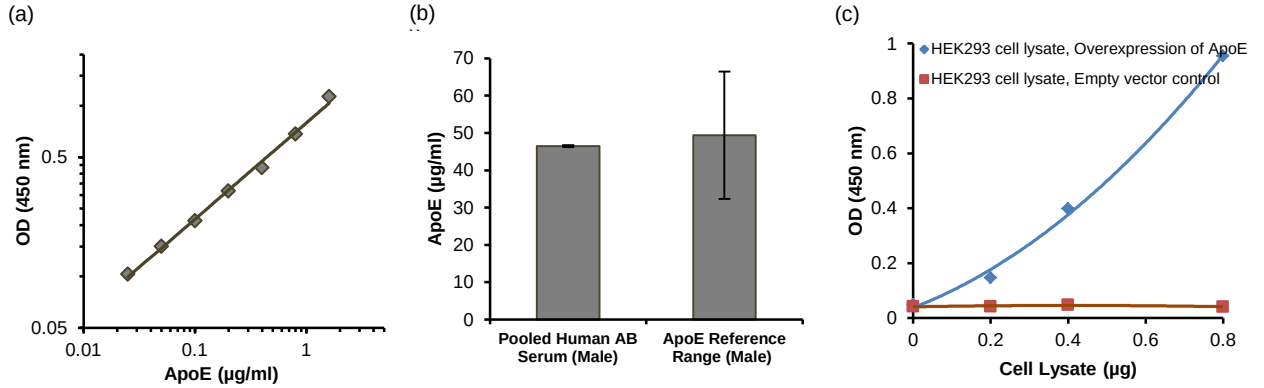


Figure: a) rh ApoE Standard Curve. This standard curve is for demonstration only. A standard curve must be run with each assay. b) Measurement of ApoE concentration in human serum. Sample of pooled human serum (male) was analyzed following the kit protocol (left) and compared to the literature values (right). C) Quantitation of ApoE in ApoE overexpressed HEK293T cell line. Cell lysate of ApoE overexpressed HEK293T cell line was analyzed and compared to Empty Vector control lysate.

IX. RELATED PRODUCTS:

ApoE2, human recombinant (4760)
ApoE3, human recombinant (4696)
ApoE4, human recombinant (4699)

ApoE Antibody (CT) (6720)
ApoE2 Antibody (5760)
ApoE4/Pan-ApoE ELISA Kit (K4699-100)

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