

**Apo E ELISA Kit –  
Biotin Detection Antibody  
Format  
96T. General Protocol**

**FIVEphoton  
Biochemicals**

**For research use only.  
Not for diagnostics.**

Part No. mAPOE-Biotin

Use this protocol as a general guide. For your assay, use the protocol that is shipped with the kit

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**Store:** 2-8°C. Expiration: 6 months after arrival.

**Safety:** Stop solution contains acid. Avoid contact and inhalation. Wear eye protection.

# Apolipoprotein E (Apo E) ELISA Kit – Biotin Detection Antibody Format

## Part No. mAPOE-Biotin

For research only. Not for diagnostic applications.

Storage: 2-8°C, expires six months after arrival

Safety: Stop solution contains acid. Avoid eye and skin contact

Species	Standard Peptide	Assay Range
Human	320 µg/ml	1 - 300 µg/ml
Rat	160 µg/ml	50ng/ml - 150 µg/ml
Mouse	96 µg/ml	30ng/ml- 90 µg/ml

**Overview:** Please familiarize yourself with this protocol prior to performing the assay. It is recommended that the researcher overviews the literature to identify optimal and detailed methods for sample preparation: The sample isolation methods discussed in this protocol serve only as a general guide. Blanks, Standards and Samples should be measured at least in duplicate to account for pipetting error.

The kit measures Mouse Apo E in sandwich ELISA format. The ELISA plate is provided pre-coated with an anti-mouse Apo E monoclonal antibody. The experimenter mixes the samples with a provided polyclonal anti-mouse Apo E antibody labeled with biotin and with a provided streptavidin-HRP solution. This sample-antibody-biotin-streptavidin-HRP mixture is then added to the ELISA plate, which is incubated for 60 min at 37°C. Unbound biotin-linked antibody is washed away, and color reagents are added that change the color of the solution to blue. The blue solution then turns yellow after addition of the stop solution. The absorbance of the resulting yellow solution at 450nm corresponds to the concentration of Mouse Apo E in the samples.

### Sample Preparation:

1. Note that samples containing  $\text{NaN}_3$  may inhibit Horse Radish Peroxidase (HRP) and the color reaction.
2. If possible, assay the sample immediately after isolation. Otherwise store samples at -20°C without free-thawing cycles.
3. **Serum:** Allow serum to clot for 10-20 minutes at room temperature. Centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatant carefully. If particulates are detected after storage, centrifuge again and collect the supernatant for assays. A significant dilution of serum in the sample diluent solution (for example 200-1000X) may be required to maintain assay specificity.
4. **Blood plasma:** In accordance with the requirements of sample collection, EDTA or sodium citrate should be used as the anti-coagulant. Add EDTA or sodium citrate and mix 10-20 minutes. Centrifuge at 2000-3000 RPM for approximately 20 minutes. Collect the supernatants carefully. If particulates are detected after storage, centrifuge again and collect the supernatant for assays. A significant dilution of plasma in the sample diluent solution (for example 200-1000X) may be required to maintain specificity of the assay.
5. **Urine:** Collect by sterile tube. Centrifuge at 2000-3000 RPM for approximately 20 minutes. Collect the supernatants

carefully. When particulates appear due to storage, centrifuge again and collect the supernatant to assay.

6. **Cell culture supernatant:** Collect in sterile tubes when examining secreted components. Centrifuge at 2000-3000 RPM for approximately 20 minutes to sediment cells. Collect the supernatants carefully.

7. **Intracellular and membrane bound components.** To assay intracellular components, use a non-denaturing cell lysis buffer to lyse membranes and solubilize cytoplasmic and membrane bound components. Add ice cold lysis buffer with protease inhibitors to cells. Keep the tube in ice for 30 min. Tap the tube several times during this period to mechanically shear membranes. Centrifuge cellular debris in a microcentrifuge at 18000 rpm for 15 min. Collect the supernatant to assay.

8. **Tissue sample:** Use a homogenization protocol in a non-denaturing buffer to release protein components. Add protease inhibitors and keep the solution ice cold during the homogenization steps. Centrifuge debris and assay the supernatant.

**Do not use denaturing cell lysis buffers such as RIPA for protein extraction.**

9. Samples can be aliquoted and stored at -80°C for later use.

10. Samples can be diluted in a sample dilution solution with the formula: 1% BSA in PBS pH 7.4.

**Table 1. Materials supplied for the 96T assay kit. Store all materials at 4°C**

1	Standard peptide	0.5ml	7	Chromogen Solution A	6ml
2	Standard diluent (1% BSA in PBS pH 7.4)	3 ml	8	Chromogen Solution B	6ml
3	Anti-mouse ApoE Coated ELISA plate	12 wellx8 strips	9	Stop Solution	6ml
4	Streptavidin-HRP	6 ml	10	Instruction Manual	1
5	30xwash solution	20 ml			
6	Anti mouse Apo E antibody labeled with biotin (detection antibody)	1ml			

**Materials required but not supplied**

1. Sample dilution buffer (1% BSA in PBS pH 7.4)
2. 37°C incubator
3. Standard absorbance plate reader
4. Precision pipettes and disposable pipette tips
5. De-ionized water
6. Disposable tubes for sample dilution
7. Absorbent paper

**Important notes and preparation for the assay**

1. Make a solution that is 1% BSA in PBS pH 7.4 for dilution of the samples (i.e." sample dilution solution").
2. Equilibrate the ELISA plate strips and solutions to room temperature for at least 30 minutes. The plate strips should be kept in a zip lock bag to prevent drying of the wells. Store unused sections of the ELISA plate in a sealed bag at 4°C.
3. Use new pipettes tips at each step to prevent contamination.
4. Do not use reagents from other ELISA kits..
5. Substrate B is light sensitive. Limit light exposure.

6. **Manual Wash Method:** Gently aspirate off the liquid in each well. Turn over the plate and pat-dry on absorbent paper. Add 100µl wash solution and repeat 5 times. Therefore 600ul wash solution is needed per well. An automatic washer can also be employed to wash the ELISA wells.

### **Assay procedures**

Blanks, standard and sample preparation: Blanks, standards and samples should be added simultaneously to wells.

**Prepare the blanks, standards and samples in a separate 96-well dish and transfer simultaneously to the ELISA plate. Do not prepare samples in the ELISA plate.**

#### **Assay procedure**

1. Standard dilutions. Use Table 2 as a general guide for dilutions of standards in the standard diluent solution. Other sequential dilutions that span the assay range can also be used. Make the dilutions in separate tubes or multi-well dishes, not in the ELISA dish.

**Table 2. Standard Dilutions (Shown for Mouse Apo E ELISA Kit as an example)**

Standard Concentration	Standard Number	Dilution Instructions
48 µg/ml	11	120µl original standard + 120µl standard diluent solution, mix
24 µg/ml	10	120µl standard No. 11 + 120µl standard diluent solution, mix
12 µg/ml	9	120µl standard No. 10 + 120µl standard diluent solution, mix
6 µg /ml	8	120µl standard No. 9 + 120µl standard diluent solution, mix
3 µg /ml	7	120µl standard No. 8 + 120µl standard diluent solution, mix
1.5 µg/ml	6	120µl standard No. 7 + 120µl standard diluent solution, mix
750 ng/ml	5	120µl standard No. 6 + 120µl standard diluent solution, mix
375 ng/ml	4	120µl standard No. 5 + 120µl standard diluent solution, mix
187 ng/ml	3	120µl standard No. 4 + 120µl standard diluent solution, mix
94 ng/ml	2	120µl standard No.3 + 120µl standard diluent solution, mix
47 ng/ml	1	120µl standard No.2 + 120µl standard diluent solution, mix

2. Blank, standard and sample preparation: (Pre-mix solutions in a separate multiwell dish and transfer the solutions simultaneously to the ELISA dish. Do not pre-mix solutions in the ELISA dish).
  - a) Blank wells: Only add chromogen solutions and stop solutions at the appropriate steps of the assay. Otherwise follow the protocol omitting the other solutions.
  - b) Standard solution wells: Add 50µl of diluted standard and 50µl Streptavidin-HRP solution (detection antibody labeled with biotin has been added in advance to the standards, therefore no biotin antibodies are added to the standards).
  - c) Sample well: Dilute 10ul sample in 30µl sample diluent solution (1% BSA in PBS pH 7.4 – made by the experimenter), then add 10µl mouse Apo E detection-biotin antibody and 50µl Streptavidin-HRP. Mix gently; do not vortex. Transfer the solutions to the ELISA dish. Seal the wells with a ziplock bag and rotate the plate gently for 60 minutes at 37°C.

3. Preparation of wash solution: Dilute the 30X wash solution to 1X with dH<sub>2</sub>O. Prepare 600µl 1X wash solution per well.
4. Wash: Carefully remove the seal plate membrane or zip lock bag that protects the wells from drying: do not cross-contaminate liquid. Aspirate off the liquid. Fill each well with 100µl wash solution; gently rotate. Aspirate the liquid after 30 seconds. Then repeat the wash procedure five times. Blot dry the plate.
5. Color development: First add 50µl chromogen solution A to each well and then add 50µl chromogen solution B to each well. Shake gently to mix solutions A and B. Incubate protected from light for 10 minutes at 37°C.
6. Stop: Add 50µl Stop Solution to each well to stop the reaction (the blue color changes into yellow).
7. Read samples at 450nm within 10 min of adding the stop solution: Set the blank well as zero, measure the absorbance (OD) of each well at 450nm.

### Data Analysis

1. Compile a standard curve using the blank standard solutions and the corresponding OD values. You may wish to calculate a linear regression equation from the standard curve to determine the concentration of your samples. Take into account dilution factors in your calculation of Mouse Apo E concentration. Alternative methods of data analysis to calculate concentrations of your samples can also be employed.

### Flow chart of the procedures

Separately prepare standards, blank and samples with detection antibody and HRP-streptavidin as required



Incubate for 1 hr at 37°C.



Wash the plate five times.



Add Chromogen solutions A and B. Incubate for 10 min at 37°C for color development.



Add stop solution



**Measure OD value at 450 nm within 10 min**