

## Datasheet for ABIN4947363 **APOA4 ELISA Kit**



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### Overview

Quantity:	96 tests
Target:	APOA4
Reactivity:	Mouse
Method Type:	Sandwich ELISA
Detection Range:	0.156 ng/mL - 10 ng/mL
Minimum Detection Limit:	0.156 ng/mL
Application:	ELISA

### Product Details

Purpose:	High Sensitive ELISA Kit for Apolipoprotein A4 (APOA4)
Sample Type:	Plasma, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	<p>This assay has high sensitivity and excellent specificity for detection of High Sensitive Apolipoprotein A4 (APOA4).</p> <p>No significant cross-reactivity or interference between High Sensitive Apolipoprotein A4 (APOA4) and analogues was observed.</p>
Sensitivity:	0.064 ng/mL
Grade:	High Sensitivity
Components:	<ul style="list-style-type: none"><li>• Pre-coated, ready to use 96-well strip plate</li></ul>

## Product Details

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- Plate sealer for 96 wells
- Standard Diluent
- Assay Diluent A
- Assay Diluent B
- Stop Solution
- Standard
- Detection Reagent A
- Detection Reagent B
- TMB Substrate
- Wash Buffer (30 x concentrate)
- Instruction manual

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### Material not included:

- Microplate reader with 450 nm filter.
- Precision single or multi-channel pipettes and disposable tips.
- Eppendorf Tubes for diluting samples.
- Deionized or distilled water.
- Absorbent paper for blotting the microtiter plate.
- Container for Wash Solution

## Target Details

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Target:	APOA4
Alternative Name:	Apolipoprotein A4 ( <a href="#">APOA4 Products</a> )
UniProt:	<a href="#">P06728</a>
Pathways:	<a href="#">Lipid Metabolism</a>

## Application Details

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Application Notes:	<ul style="list-style-type: none"><li>• Limited by the current condition and scientific technology, we cannot completely conduct the comprehensive identification and analysis on the raw material provided by suppliers. So there might be some qualitative and technical risks to use the kit.</li><li>• The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments. Please make sure that sufficient samples are available.</li><li>• Kits from different batches may be a little different in detection range, sensitivity and color developing time.</li><li>• Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.</li><li>• Protect all reagents from strong light during storage and incubation. All the bottle caps of reagents should be covered tightly to prevent the evaporation and contamination of microorganism.</li></ul>
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- There may be some foggy substance in the wells when the plate is opened at the first time. It will not have any effect on the final assay results. Do not remove microtiter plate from the storage bag until needed.
- Wrong operations during the reagents preparation and loading, as well as incorrect parameter setting for the plate reader may lead to incorrect results. A microplate plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 O.D. or greater at 450 ± 10nm wavelength is acceptable for use in absorbance measurement. Please read the instruction carefully and adjust the instrument prior to the experiment.
- Even the same operator might get different results in two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled. Furthermore, a preliminary experiment before assay for each batch is recommended.
- Each kit has been strictly passed Q.C test. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipments. Intra-assay variance among kits from different batches might arise from above factors, too.
- Kits from different manufacturers for the same item might produce different results, since we have not compared our products with other manufacturers.

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Comment:

Information on standard material:

The standard might be recombinant protein or natural protein, that will depend on the specific kit. Moreover, the expression system is E.coli or yeast or mammal cell. There is 0.05% proclin 300 in the standard as preservative.

Information on reagents:

The stop solution used in the kit is sulfuric acid with concentration of 1 mol/L. And the wash solution is TBS. The standard diluent contains 0.02 % sodium azide, assay diluent A and assay diluent B contain 0.01% sodium azide. Some kits can contain is BSA in them.

Information on antibodies:

The provided antibodies and their host vary in different kits.

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Sample Volume:

100 µL

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Assay Time:

3 h

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Plate:

Pre-coated

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Protocol:

The test principle applied in this kit is Sandwich enzyme immunoassay. The microtiter plate provided in this kit has been pre-coated with an antibody specific to High Sensitive Apolipoprotein A4 (APOA4). Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated antibody specific to High Sensitive Apolipoprotein A4 (APOA4). Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate

well and incubated. After TMB substrate solution is added, only those wells that contain High Sensitive Apolipoprotein A4 (APOA4), biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of  $450\text{nm} \pm 10\text{nm}$ . The concentration of High Sensitive Apolipoprotein A4 (APOA4) in the samples is then determined by comparing the O.D. of the samples to the standard curve.

### Reagent Preparation:

1. Bring all kit components and samples to room temperature (18-25 °C) before use. If the kit is not used up all at once, remove only the strips and reagents for the current experiment and leave the remaining strips and reagents in the desired condition.
2. **Standard** - Reconstitute the Standard with 0.5mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is 1025ng/mL. Prepare 7 tubes containing 0.25mL Standard Diluent and produce a double dilution series. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 10ng/mL, 5ng/mL, 2.5ng/mL, 1.25ng/mL, 0.625ng/mL, 0.312ng/mL, 0.156ng/mL, and the last tubes with Standard Diluent is the blank as 0ng/mL.
3. **Detection Reagent A** and **Detection Reagent B** - Spin or centrifuge the stock of Detection Reagent A and B briefly before use. Dilute to working concentration (1:100) with Assay Diluent A or B, respectively.
4. **Wash Solution** - Dilute 20 mL of Wash Solution Concentrate (30x) with 580 mL of deionized or distilled water to make 600 mL of Wash Solution (1x).
5. **TMB Substrate** - Aspirate the required amount of solution with sterile tip and do not return the residual solution back into the vial.

### Note:

1. Serial dilution directly in the wells is not recommended.
2. Prepare standard within 15 minutes before assay. Do not dissolve the reagents directly at 37 °C.
3. Detection Reagent A and B are sticky solutions, so pipette them slowly to reduce volume errors.
4. Reconstitute Standard or working solutions of Detection Reagent A and B carefully according to instructions, avoiding foaming and mixing gently until crystals are completely dissolved. To minimize inaccuracy caused by pipetting, use small volumes and ensure pipettes are calibrated. It is recommended to aspirate more than 10  $\mu\text{L}$  for one-time pipetting.
5. The reconstituted Standard, Detection Reagent A and B can only be used once.
6. When crystals have formed in the Wash Solution concentrate (30x), warm it to room temperature and mix gently until the crystals are completely dissolved.
7. Contaminated water or preparation containers affect the detection result.

### Sample Preparation:

- We are only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.

## Application Details

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- Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments. Sample should be diluted by 0.01mol/L PBS(PH=7.0-7.2).
- If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
- Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts from certain chemicals.
- Due to the possibility of mismatching between antigen from other origin and antibody used in our kits (e.g. antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
- Influenced by the factors including cell viability, cell number or sampling time, samples from cell culture supernatant may not be detected by the kit.
- Fresh samples without long time storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

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### Assay Procedure:

1. Prepare all reagents, samples and standards,
2. Add 100µL standard or sample to each well. Incubate 2 hours at 37 °C,
3. Aspirate and add 100µL prepared Detection Reagent A. Incubate 1 hour at 37 °C,
4. Aspirate and wash 3 times,
5. Add 100µL prepared Detection Reagent B. Incubate 30 minutes at 37 °C,
6. Aspirate and wash 5 times,
7. Add 90µL Substrate Solution. Incubate 10-20 minutes at 37 °C,
8. Add 50µL Stop Solution. Read at 450nm immediately.

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### Assay Precision:

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level High Sensitive Apolipoprotein A4 (APOA4) were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level High Sensitive Apolipoprotein A4 (APOA4) were tested on 3 different plates, 8 replicates in each plate.

$$CV(\%) = SD/\text{mean} \times 100$$

Intra-Assay: CV<10%

Inter-Assay: CV<12%

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### Restrictions:

For Research Use only

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## Handling

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### Precaution of Use:

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

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### Handling Advice:

The stability of kit is determined by the loss rate of activity. The loss rate of this kit is less than

## Handling

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5 % within the expiration date under appropriate storage condition.

To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.

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Storage: 4 °C

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Storage Comment:

- For unopened kit: All the reagents should be kept according to the labels on vials. The Standard, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20°C upon receipt while the others should be at 4°C.
- For opened kit: When the kit is opened, the remaining reagents still need to be stored according to the above storage condition. Besides, please return the unused wells to the foil pouch containing the desiccant pack, and reseal along entire edge of zip-seal.  
Note: It is highly recommended to use the remaining reagents within 1 month provided this is within the expiration date of the kit.
- For ELISA kit, 1 day storage at 37°C can be considered as 2 months at 4°C, which means 3 days at 37°C equaling 6 months at 4°C.

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Expiry Date: 6 months