



**User's Manual**

# Human Apolipoprotein DELISA Kit (APOD)

REF

DEIA-APOD02



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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

The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of APOD in human serum, plasma, cell culture supernatants, cell lysates, tissue homogenates and other biological fluids.

### Principles of Testing

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human APOD has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any APOD present is bound by the immobilized antibody. After washing away any unbound substances, and then a detection antibody specific for APOD is added to the wells and binds to the combination of capture antibody APOD in sample. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Following incubation and wash steps, a substrate solution is added to the wells and color develops in proportion to the amount of APOD bound in the initial step. The color development is stopped and the absorbance is measured.

### Reagents And Materials Provided

1. Antibody Coated Plate, 8x12. Put the unused slats back in the aluminum foil bag with the desiccant and reseal them. They can be stored at 2-8°C for 1 month.
2. Standard Lyophilized, 2 vials. It is not recommended to use again after redissolving.
3. Concentrated Biotin Conjugate Antibody (100x), 1 x 120ul. Store at 2-8°C for 1 month
4. Streptavidin-HR P Concentrated (100x), 1 x 120ul. Store at 2-8°C for 1 month
5. Standard/Sample Diluent (R1), 1 x 20mL. Store at 2-8°C for 1 month
6. Biotin-Conjugate Antibody Diluent (R2), 1 x 12mL. Store at 2-8°C for 1 month
7. Streptavidin-HRP Diluent(R3), 1 x 12mL. Store at 2-8°C for 1 month
8. Wash Buffer(20x), 1 x 30mL. Store at 2-8°C for 1 month
9. TMB Substrate, 1 x 12mL. Store at 2-8°C for 1 month
10. Stop Solution, 1 x 6mL. Store at 2-8°C for 1 month

### Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 630 nm or 570 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirt bottle, manifold dispenser, or automated microplate washer.
5. Incubator.
6. Test tubes for dilution of standards and samples.

## Storage

Unopened kits can be stored at 2-8°C for 1 year, and opened products must be used within 1 month.

## Specimen Collection And Preparation

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated. Samples containing the correlated IgG as in this kit may interfere with this assay.

- 1. Cell Culture Supernatant:** Remove particulates by centrifugation. Assay immediately or aliquot and store samples at  $\leq -20^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.
- 2. Serum:** Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at  $\leq -20^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.
- 3. Plasma:** Collect plasma using EDTA or Heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at  $\leq -20^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles. (Note: Citrate plasma has not been validated for use in this assay.)
- 4. Cell Lysates:** Cells need to be lysed before assaying according to the following directions. Adherent cells should be washed by cold PBS gently, and then detached with trypsin, and collected by centrifugation at 1,000xg for 5 minutes (suspension cells can be collected by centrifugation directly). Wash cells three times in cold PBS. Resuspend cells in fresh lysis buffer with concentration of  $10^7$  cells/mL. If it is necessary, the cells could be subjected to ultrasonication till the solution is clarified. Centrifuge at 1,500xg for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or aliquot and store at  $\leq -20^{\circ}\text{C}$ .
- 5. Tissue homogenates:** The preparation of tissue homogenates varies depending upon tissue type. Tissues are rinsed in ice-cold PBS to remove excess blood thoroughly and weigh before homogenization. Mince the tissues to small pieces and homogenized them in fresh lysis buffer with a glass homogenizer on ice or using Micro Tissue Grinders. Different lysis buffer should be chosen based on subcellular location of the target protein (e.g. 1mL lysis buffer is added in 200mg tissue sample). The resulting suspension is sonicated with an ultrasonic cell disrupter till the solution is clarified. Then, the homogenates are centrifuged for 5 minutes at 10,000 x g. Collect the supernatants and assay immediately or aliquot and store at  $\leq -20^{\circ}\text{C}$ .
- 6. Other biological fluids:** Centrifuge samples for 20 minutes at 1,000xg. Collect the supernatants and assay immediately or store samples in aliquot at  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$  for later use. Avoid repeated freeze-thaw cycles.

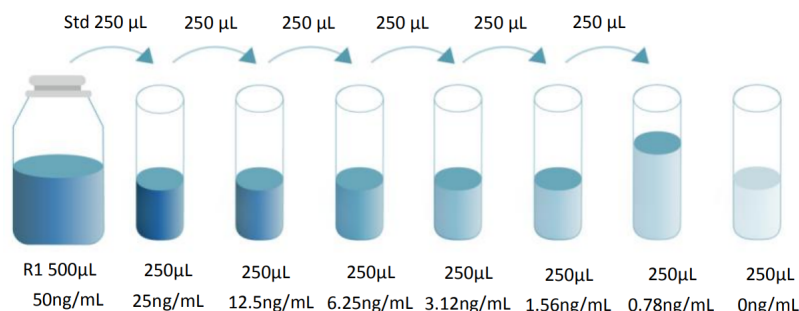
**Note: It is suggested that all samples in one experiment be collected at the same time of the day. Avoid hemolytic and hyperlipidemia sample for serum and plasma.**

## Reagent Preparation

Bring all reagents to room temperature before use. If crystals have formed in the concentrate, Bring the reagent to room temperature and mix gently until the crystals have completely dissolved.

- 1. Standard:** Reconstitute the Standard Lyophilized with 0.5 mL Standard/Sample Diluent(R1). This reconstitution produces a stock solution of 50ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Use the

50ng/mL standard stock to produce a dilution series (below) with Standard/Sample Diluent(R1). Mix each tube thoroughly and change pipette tips between each transfer (recommended concentration for standard curve: 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0ng/mL). Use diluted standards within 60 minutes of preparation.



**2. Working Biotin Conjugate Antibody:** Dilute 1:100 of Concentrated Biotin Conjugate Antibody (100x) with Biotin-Conjugate Antibody Diluent (R2) before use, for example: Add 20 µL of Concentrated Biotin Conjugate Antibody (100x) to 1980 µL Biotin-Conjugate Antibody Diluent (R2) to prepare 2000 µL Working Biotin Conjugate Antibody Buffer.

**3. Working Streptavidin-HRP:** Dilute 1:100 of Concentrated Streptavidin-HRP (100x) with Streptavidin-HRP Diluent (R3) before use, for example: Add 20 µL of Concentrated Streptavidin-HRP (100x) to 1980 µL Streptavidin-HRP Diluent (R3) to prepare 2000 µL Working Streptavidin-HRP Buffer.

**4. Wash Buffer:** If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 1:20 with double distilled or deionized water before use, for example : Add 20 mL of Wash Buffer Concentrate to 380 mL of deionized or distilled water to prepare 400 mL of Wash Buffer.

## Assay Procedure

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

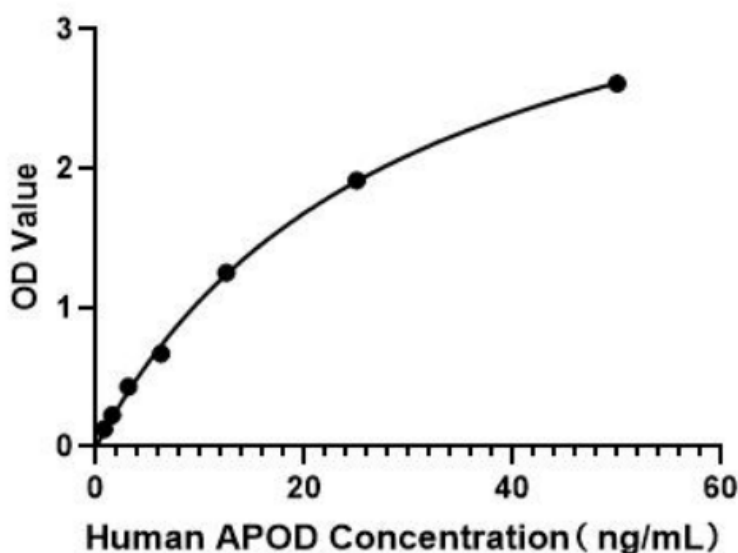
1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal properly.
3. Add wash buffer 350 µL/well, aspirate each well after holding 40 seconds, repeating the process two times for a total of three washes.
4. Add 100 µL Standard/sample Diluent (R1) in a blank well.
5. Add 100 µL different concentration of standard or sample in other wells, Cover with the adhesive strip provided. Incubate for 2 hours at 37°C. record the plate layout of standards and sample assay.
6. Prepare the Concentrated Biotin Conjugate Antibody (100x) Working Solution 15 minutes early before use.
7. Repeat the aspiration/wash as in step 3.
8. Add 100 µL Working Biotin Conjugate Antibody in each well, cover with new adhesive Sealer provided. Incubate for 1 hour at 37°C.
9. Prepare the Streptavidin-HRP Concentrated (100x) Working Solution 15 minutes early before use.
10. Repeat the aspiration/wash as in step 3.

11. Add 100  $\mu$ L Working Streptavidin-HRP in each well, cover with new adhesive Sealer provided. Incubate for 0.5 hour at 37°C.
12. Repeat the aspiration/wash as in step 3.
13. During the incubation, turn on the microplate reader to warm up for 30 minutes before measuring.
14. Add 100  $\mu$ L TMB Substrate to each well. Incubate for 15-20 minutes at 37°C .Protect from light.
15. Add 50  $\mu$ L Stop Solution, determine the optical density of each well within 5 minutes, using a Microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may cause higher value and less accurate result.

## Calculation

1. Average the duplicate readings for each standard, control and sample, and subtract the average zero standard optical density (O.D.).
2. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the APOD concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.
3. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## Typical Standard Curve



The standard curves are provided for demonstration only. A standard curve should be generated for each set of APOD assayed.

## Precision

### Intra-plate Precision

3 samples with low, middle and high level APOD were tested 20 times on one plate, respectively.

Intra-Assay: CV<10%

### Inter-plate Precision

3 samples with low, middle and high level APOD were tested on 3 different plates, 20 replicates in each plate.

Inter-Assay: CV<15%

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/mL)	7	20.3	45.8	9.3	30.5	50
Standard deviation	0.15	0.89	1.56	0.54	2.38	3.05
CV (%)	2.1	4.4	3.4	5.8	7.8	6.1

## Detection Range

0.78-50ng/mL

## Sensitivity

The minimum detectable dose (MDD) of APOD typically less than 0.36ng/mL. The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

## Specificity

This method has high sensitivity and specificity for APOD detection, and there is no obvious cross-reaction or interference between APOD and analogues.

### Note:

Due to the limitations of existing technology and knowledge, it is impossible for us to complete the cross-reaction detection between APOD and all analogues, so the cross-reaction may still exist.

## Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of APOD and

their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

/	/	Cell Culture Media (n=5)	Serum (n=5)
1:2	Average of Expected (%)	94	87
	Range (%)	85-103	80-94
1:4	Average of Expected (%)	95	92
	Range (%)	90-100	81-103
1:8	Average of Expected (%)	105	88
	Range (%)	90-119	82-94
1:16	Average of Expected (%)	90	98
	Range (%)	80-100	80-116

## Recovery

Matrices listed below were spiked with certain level of APOD and the recovery rates were calculated by comparing the measured value to the expected amount of APOD in samples.

Sample	Average Recovery (%)	Range (%)
Cell Culture Media (n=5)	90	80-95
Serum (n=5)	92	90-100

## Precautions

- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- Reagents may be harmful, if ingested, rinse it with an excess amount of tap water.
- Stop Solution contains strong acid. Wear eye, hand, and face protection.
- Please perform simple centrifugation to collect the liquid before use.
- Do not mix or substitute reagents with those from other lots or other sources.
- Adequate mixing is particularly important for good result. Use a mini-vortexer at the lowest frequency.
- Mix the sample and all components in the kits adequately, and use clean plastic container to prepare all diluents.

9. Both the sample and standard should be assayed in duplicate, and reagents should be added in sequence in accordance with the requirement of the specification.
10. Reuse of dissolved standard is not recommended.
11. The kit should not be used beyond the expiration date on the kit label.
12. The kit should be away from light when it is stored or incubated.
13. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma, and other biological fluids in accordance with NCCLS regulations.
14. To avoid cross contamination, please use disposable pipette tips.
15. Please prepare all the kit components according to the Specification. If the kits will be used several times, please seal the rest strips and preserve with desiccants. Do use up within 2 months.
16. This assay is designed to eliminate interference by other factors present in biological samples.
17. Until all factors have been tested in this assay, the possibility of interference cannot be excluded.

