



# AHSG (Human) ELISA Kit

Catalog Number KA6405

96 Assays

Version: 01

Intended for research use only

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## **Introduction**

### **Intended Use**

The AHSG (Human) ELISA Kit is a sandwich ELISA designed for the quantitative measurement of Fetuin A in serum, plasma, tissue homogenates and biological fluids.

### **Background**

Fetuin A is a glycoprotein which is also called as alpha-2-HS-glycoprotein. It is abundant in fetal blood and is a hepatocyte derived protein. It serves as a transport protein for calcium and phosphate, inhibits extrasosseous calcification and plays an important role in bone mineralization, insulin resistance and breast tumor cell proliferative signaling. It is downregulated in Alzheimer's disease patients and could serve as a potential biomarker.

### **Principle of the Assay**

It is based on the principle of sandwich ELISA. The capture antibody is pre-coated on 96-well plates. The standards, test samples and biotin conjugated detection antibody are added to the wells subsequently, and washed with wash buffer. HRP-Streptavidin is added and unbound conjugates are washed away with wash buffer. The HRP enzymatic reaction is detected using TMB-substrate. Finally, an acidic stop solution terminates the enzymatic reaction. The color developed is directly proportional to the amount of Fetuin A in the sample.

### **Features**

- ✓ Detection range: 0.781 – 50 ng/mL
- ✓ Sensitivity: 0.469 ng/mL
- ✓ Assay Precision: Intra-Assay CV < 8% and Inter-Assay CV < 10%
- ✓ Recovery Rate: 90-105% for serum, 90-104% for EDTA plasma and 85-105% for heparin plasma
- ✓ There is no significant cross-reactivity or interference between Fetuin A and analogues.

## General Information

### Materials Supplied

List of component

Component	Amount	Storage Temp.
ELISA Microplate	1 plate (8 X 12 strips)	4°C
Lyophilized Standard (50 ng)	2 vials	4°C
Sample/Standard Dilution Buffer	20 mL	4°C
Biotin-labeled Antibody (Concentrated)	120 µL	4°C (Avoid light)
Antibody Dilution Buffer	10 mL	4°C
HRP-Streptavidin Conjugate (SABC)	120 µL	4°C (Avoid light)
SABC Dilution Buffer	10 mL	4°C
TMB Substrate	10 mL	4°C (Avoid light)
Stop Solution	10 mL	4°C
Wash Buffer (25X)	30 mL	4°C
Plate Sealers	5	4°C

### Storage Instruction

The entire kit can be stored at 4°C for up to 6 months.

### Materials Required but Not Supplied

- ✓ Microplate reader capable of measuring absorbance at 450 nm
- ✓ 37°C incubator
- ✓ Precision pipettes with disposable tips
- ✓ Distilled or deionized water
- ✓ Clean Eppendorf tubes for preparing standards or sample dilutions
- ✓ Absorbent paper

### Precautions for Use

For research use only. Not to be used on humans.

## Assay Protocol

### Reagent Preparation

*Note: Bring all reagents and samples to room temperature (RT) for 20 min before use.*

*Before using the kit, spin tubes and bring down all components to the bottom of tubes.*

*After opening and before using, keep plate dry.*

*Don't let microplate dry during the assay, as dry plate will inactivate active components on plate.*

- ✓ **Wash Buffer:** Dilute 25X Wash Buffer to 1X by adding 30 mL of 25X Wash Buffer and make up the volume to 750 mL with deionized/distilled water. If crystals are present in the 25X Wash Buffer, warm it in a water bath at 40°C. Mix it gently. The solution must be cooled to RT before use. Store unused solution at 4°C.
- ✓ **Standard Preparation:**
  - Add 1 mL Sample Dilution Buffer into one Standard tube (labeled as zero tube). Keep the tube at room temperature for 10 minutes. Mix thoroughly.
  - Label 7 tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3 mL of the Sample Dilution Buffer into each tube. Add 0.3 mL of the above Standard solution (from zero tube) into 1st tube and mix them thoroughly.
  - Transfer 0.3 mL from 1st tube to 2nd tube and mix them thoroughly. Transfer 0.3 mL from 2nd tube to 3rd tube and mix them thoroughly, and so on. Sample Dilution Buffer was used for the blank control. (Note: Please use Standard Solutions within 2 hours of preparation).

Note: If the standard tube concentration is higher than the range of the kit, please dilute and use.
- **Biotin-labeled Antibody working solution:** Prepare this working stock 1 h prior to the start of the experiment. Calculate the total volume of the working solution: 0.1 mL/well x quantity of wells. Add 0.1-0.2 mL to the total volume. Dilute the Biotin-labeled antibody with Antibody Dilution Buffer at 1:100. Mix thoroughly.
- **HRP-Streptavidin Conjugate (SABC):** Prepare this working stock 30 min prior to the start of the experiment. Calculate the total volume of the working solution: 0.1 mL/well x quantity of wells. Add 0.1-0.2 mL to the total volume. Dilute the SABC with SABC Dilution Buffer at 1:100. Mix thoroughly.

## **Sample Preparation**

Sample type: Human Serum, Plasma, Tissue lysates and other biological fluids

*Note: Isolate the test samples soon after collecting, then, analyze immediately. Alternatively, aliquot and store at -20 °C (≤ 1 month) or -80 °C (≤ 2 months). Avoid multiple freeze-thaw cycles. Hemolytic samples are not suitable for this assay*

- Serum: Place whole blood sample at RT for 2 h or keep at 4 °C overnight and centrifuge for 20 min at approximately 1000xg. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non- pyrogenic, and endotoxin free.
- Plasma: Collect plasma using EDTA-Na<sub>2</sub> or heparin as an anticoagulant. Centrifuge samples for 15 min at 1000xg at 4°C within 30 min of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis and high cholesterol samples.
- Tissue homogenates: As hemolytic blood may affect the assay result, it is necessary to remove residual blood by washing tissue with pre-cooled PBS buffer (0.01 M, pH 7.4). Mince tissue after weighing it and homogenize it in PBS (the volume depends on the weight of the tissue). Normally, 9 mL PBS would be appropriate for 1 gram tissue pieces. Some protease inhibitors are recommended to be added to the PBS. Homogenize using a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 min at 5000xg to get the supernatant. The total protein concentration can be determined by BCA kit and the total protein concentration of each well sample should not exceed 0.3 mg/mL.
- Cell culture supernatant: Centrifuge supernatant for 20 min at 1000xg at 4 °C to remove insoluble impurities and cell debris. Collect the clear supernatant and carry out the assay immediately.
- Cell Culture Lysate: Commercial RIPA kits are recommended. Follow the instructions provided. Generally, 0.5 mL RIPA lysis buffer would be appropriate for 2x10<sup>6</sup> cells, DNA must be removed. The total protein concentration can be determined by BCA kit and the total protein concentration of each well sample should not exceed 0.3 mg/mL.
- Other Biological fluids: Centrifuge samples for 20 min at 1000xg at 4°C. Collect the supernatant and carry out the assay immediately.

*Note: End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit. The matrix components in the sample may affect the test results. Please dilute the sample ½ with Sample Dilution Buffer before testing.*

## **Assay Procedure**

*Note: When diluting samples and reagents, the solutions must be mixed completely and evenly.*

*Before adding TMB into wells, equilibrate TMB Substrate for 30 min at 37 °C.*

*It is recommended that all standards and samples be run at least in duplicate.*

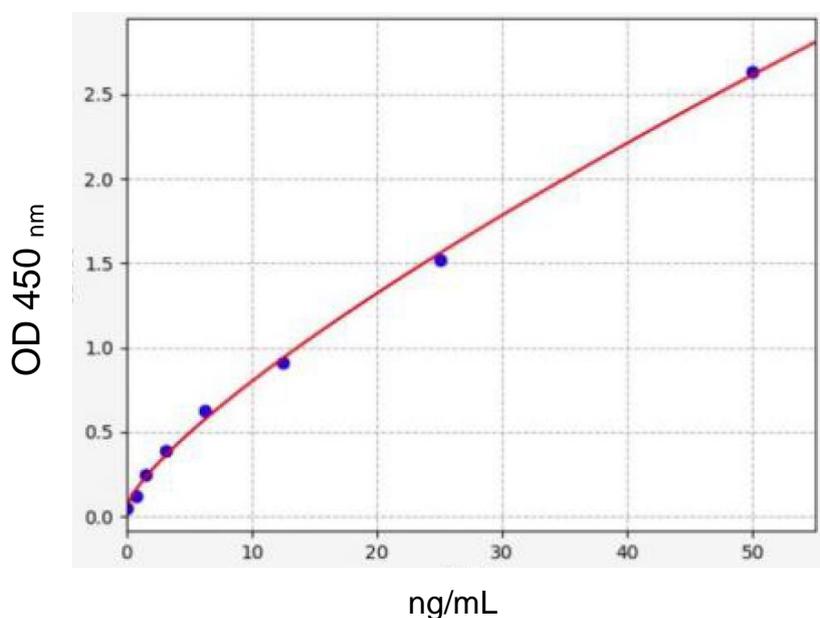
*A standard curve must be run with each assay.*

1. Prepare all reagents, samples (diluted ½ with Sample Dilution Buffer) and standards as instructed in section Reagent and Sample Preparation.
2. Wash plate 2 times with 1X Wash Buffer before adding standard, sample (diluted ½ with Sample Dilution Buffer) and control (blank) wells.
3. Add 100 µL of each standards or samples into appropriate wells. Seal the plate with a cover and incubate for 1.5 hours at 37 °C.
4. Remove the cover and discard the plate content. Wash the plate 2 times with 1X Wash Buffer without letting the wells get completely dry.
5. Add 100 µL of Biotin-labelled antibody work solution into the above wells. Seal the plate and incubate at 37 °C for 60 min.
6. Discard the solution and wash 3 times with 1X Wash Buffer. Wash by filling each well with Wash Buffer (350 µL) using a multichannel pipette or autowasher. Let it soak for 1-2 min, and then remove all residual wash-liquid from the wells by aspiration. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Tap the plate on absorbent filter papers or other absorbent materials. Note: Washing process is very important. If washing is not done well, it may cause a false positive and high background.
7. Add 100 µL of SABC working solution into each well, cover the plate and incubate at 37 °C for 30 min.
8. Discard the solution and wash 5 times with 1X Wash Buffer as described in step 6.
9. Add 90 µL of TMB substrate into each well, cover the plate and incubate at 37 °C in dark within 10-20 min. (Note: The reaction time can be shortened or extended according to the actual color change, but not more than 30 min. The reaction can be terminated when apparent gradient appears in standard wells).
10. Add 50 µL of Stop Solution to each well. Read the absorbance at 450 nm within 20 min using a Microplate Reader.

## Data Analysis

### Calculation of Results

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of blank well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) Vs the respective concentration of the standard solution (X). The target concentration of the samples can be interpolated from the standard curve. Professional software can also be used for the calculations. If the samples measured are diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.



Typical Standard Curve and OD values: These standard curves are for demonstration only.

A standard curve must be run with each assay.

STD. (ng/mL)	OD-1	OD-2	Average	Corrected
0	0.049	0.051	0.050	0.000
0.781	0.121	0.125	0.123	0.073
1.562	0.244	0.252	0.248	0.198
3.125	0.380	0.392	0.386	0.336
6.25	0.619	0.637	0.628	0.578
12.5	0.898	0.924	0.911	0.861
25	1.498	1.542	1.520	1.470
50	2.592	2.668	2.630	2.580

### Recovery

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

Matrix	Recovery Range (%)	Average (%)
Serum (n=5)	90-105	96
EDTA Plasma (n=5)	90-104	99
Heparin Plasma (n=5)	85-105	92

### Linearity

Linearity of the assay kit was determined by spiking samples with appropriate concentration of Fetuin A and their serial dilutions. The results are represented as percentage of calculated concentration to the expected value.

Sample	1:2	1:4	1:8
Serum (n=5)	86-105%	88-104%	85-97%
EDTA Plasma (n=5)	87-100%	92-99%	85-93%
Heparin Plasma (n=5)	84-97%	83-98%	82-96%

**Resources**

**Plate Layout**

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