



User's Manual

Human Fetuin A ELISA

For detection of Fetuin A in tissue lysates or cell culture supernates.



Storage:

BE69021

96

2–8°C

RUO

For Research Use Only – Not for Use in Diagnostic Procedures.

1 INTRODUCTION

1.1 Intended Use

For quantitative detection of Fetuin A in tissue lysates or cell culture supernates. The significance of detection in human serum, plasma, body fluids is undetermined. For research use only, not for use in diagnostic procedures.

1.2 Background

Fetuin-A, also known as Alpha-2-HS-glycoprotein (AHSG) is a protein that in humans is encoded by the AHSG gene. This gene spans approximately 8.2 kb and contains 7 exons. Fetuin-A belongs to the fetuin class of plasma binding proteins and is more abundant in fetal than adult blood. It is present in the serum, and synthesized by hepatocytes. It may play some role in the metabolism of bone because of its high affinity for calcium. Fetuin-A is a potent inhibitor of pathological calcification. In the test tube Fetuin-A can bind multiple ligands and therefore has been claimed to be involved in several functions, such as endocytosis, brain development and the formation of bone tissue.

2 PRINCIPLE OF THE TEST

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Anti-Fetuin A polyclonal antibody was pre-coated onto 96-well plates. And the biotin conjugated anti-Fetuin A polyclonal antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and wash with wash buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the Fetuin A amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of Fetuin A can be calculated.

3 WARNINGS AND PRECAUTIONS

1. This kit is for research use only, not for use in diagnostic procedures.
2. Before the experiment, centrifuge each kit component for several minutes to bring down all reagents to the bottom of tubes.
3. It is recommended to measure each standard and sample in duplicate.
4. Do NOT let the plate completely dry at any time! Since the dry condition can inactivate the biological material on the plate.
5. Do not reuse pipette tips and tubes to avoid cross contamination.
6. Do not use the expired components or the components from different lot numbers.
7. To avoid the marginal effect of plate incubation for temperature differences (the marginal wells always get stronger reaction), it is recommended to equilibrate the ABC working solution and TMB substrate for at least 30 min at 37° before adding to wells.
8. The TMB substrate (Kit Component 8) is colorless and transparent before use. If not, please contact us for replacement.

4 REAGENTS

4.1 Reagents provided

1. One 96-well plate pre-coated with anti-human Fetuin A antibody
2. Lyophilized human Fetuin A standards: 2 tubes (50ng / tube)
3. Sample / Standard diluent buffer: 30ml
4. Biotin conjugated anti-human Fetuin A antibody (Concentrated): 130µl. Dilution: 1:100
5. Antibody diluent buffer: 12ml
6. Avidin-Biotin-Peroxidase Complex (ABC) (Concentrated): 130µl. Dilution: 1:100
7. ABC diluent buffer: 12ml
8. TMB substrate: 10ml
9. Stop solution: 10ml
10. Wash buffer(25X): 30ml

4.2 Materials required but not provided

1. 37° incubator
2. Microplate reader (wavelength: 450nm)
3. Precise pipette and disposable pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5ml of Eppendorf tubes
7. Plate cover
8. Absorbent filter papers
9. Plastic or glass container with volume of above 1L

4.3 Storage Conditions / Expiration

Store at 4°C for frequent use, at -20°C for 8 months.

4.4 Preparation of sample and reagents

1. Sample

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20° for long term. Avoid multiple freeze-thaw cycles.

✧ **Tissue lysate, body fluids and cell culture supernatants:** Centrifuge to remove precipitate, analyze immediately or aliquot and store at -20°C

✧ **Serum:** Coagulate the serum at room temperature (about 4 hours). Centrifuge at approximately 1000 x g for 15 min. Analyze the serum immediately or aliquot and store at -20°C.

✧ **Plasma:** Collect plasma with citrate, heparin or EDTA as the anticoagulant. Centrifuge for 15 min at 1000 x g within 30 min of collection. For eliminating the platelet effect, suggesting that further centrifugation for 10 min at 2-8°C at 10000 x g. Analyze immediately or aliquot and store frozen at -20°C.

Note: 1. Coagulate blood samples completely, then, centrifuge, and avoid hemolysis and particles.

2. NaN₃ can not be used as test sample preservative, since it is the inhibitor for HRP.

>> Sample Dilution Guideline

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 falls the optimal detection range of the kit. Dilute the sample with the provided diluent buffer, and several trials may be necessary in practice. The test sample must be well mixed with the diluent buffer.

✧ **High target protein concentration (500-5000 ng/ml):** Dilution: 1:100. i.e. Add 1 µl of sample into 99 µl of Sample / Standard diluent buffer (Kit Component 3).

✧ **Medium target protein concentration (50-500 ng/ml):** Dilution: 1:10. i.e. Add 10 µl of sample into 90 µl of Sample / Standard diluent buffer (Kit Component 3).

✧ **Low target protein concentration (780-50,000pg/ml):** Dilution: 1:2. i.e. Add 50 µl of sample into 50 µl of Sample / Standard diluent buffer (Kit Component 3).

✧ **Very low target protein concentration (≤780pg/ml):** Unnecessary to dilute, or dilute at 1:2.

2. Wash buffer

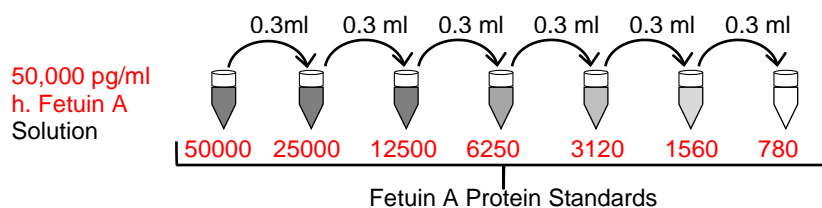
Dilute the concentrated Wash buffer 25-fold (1:25) with distilled water (i.e. add 30ml of concentrated wash buffer into 720ml of distilled water).

3. Standard

Reconstitution of the Lyophilized human Fetuin A standard (Kit Component 2): standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of standard are included in each kit. Use one tube for each experiment. (**Note: Do not dilute the standard directly in the plate**)

a. 50,000 pg/ml of standard solution: Add 1 ml of Sample / Standard diluent buffer (Kit Component 3) into one Standard (Kit Component 2) tube, keep the tube at room temperature for 10 min and mix thoroughly.

b. 25,000 pg/ml → 780 pg/ml of standard solutions: Label 6 Eppendorf tubes with 25,000 pg/ml, 12500 pg/ml, 6250 pg/ml, 3120 pg/ml, 1560 pg/ml, 780 pg/ml, respectively. Aliquot 0.3 ml of the Sample / Standard diluent buffer (Kit Component 3) into each tube. Add 0.3 ml of the above 20,000 pg/ml standard solution into 1st tube and mix thoroughly. Transfer 0.3 ml from 1st tube to 2nd tube and mix thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube and mix thoroughly, and so on.



Note: The standard solutions are best used within 2 hours. The 50,000 pg/ml standard solution should be used within 12 hours. Or store at -20° for up to 48 hours. Avoid repeated freeze-thaw cycles.

4. Preparation of Biotin conjugated anti-human Fetuin A antibody (Kit Component 4) working solution: prepare no more than 2 hours before the experiment.

a. Calculate the total volume of the working solution: 0.1 ml / well x quantity of wells. (Allow 0.1-0.2 ml more than the total volume)

b. Dilute the Biotin conjugated anti-human Fetuin A antibody (Kit Component 4) with Antibody diluent buffer (Kit Component 5) at 1:100 and mix thoroughly. i.e. Add 1 µl of Biotin conjugated anti-human Fetuin A antibody into 99 µl of Antibody diluent buffer.

5. Preparation of Avidin-Biotin-Peroxidase Complex (ABC) (Kit Component 6) working solution: prepare no more than 1 hour before the experiment.

a. Calculate the total volume of the working solution: 0.1 ml / well x quantity of wells. (Allow 0.1-0.2 ml more than the total volume)

b. Dilute the Avidin-Biotin-Peroxidase Complex (ABC) (Kit Component 6) with ABC diluent buffer (Kit Component 7) at 1:100 and mix thoroughly. i.e. Add 1 µl of Avidin-Biotin-Peroxidase Complex (ABC) into 99 µl of ABC diluent buffer.

5 ASSAY PROCEDURE

5.1 General Remarks

Before adding to wells, equilibrate the ABC working solution and TMB substrate (Kit Component 8) for at least 30 minutes at 37°. It is recommended to plot a standard curve for each test.

5.2 Test Procedure

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. It is recommended to measure each standard and sample in duplicate.
2. Aliquot 0.1ml of 50,000 pg/ml, 25,000 pg/ml, 12500 pg/ml, 6250 pg/ml, 3120 pg/ml, 1560 pg/ml, 780 pg/ml standard solutions into the standard wells.
3. Add 0.1 ml of Sample / Standard diluent buffer (Kit Component 3) into the control (zero) well.
4. Add 0.1 ml of properly diluted sample (human serum, plasma, body fluids, tissue lysates or cell culture supernatants) into test sample wells.
5. Seal the plate with a cover and incubate at 37°C for 60 min.
6. Remove the cover and discard the plate content, clap the plate on the absorbent filter papers or other absorbent material. **Do NOT let the wells completely dry at any time. Do not wash plate!**
7. Add 0.1 ml of Biotin conjugated anti-human Fetuin A antibody work solution into the above wells (standard, test sample & zero wells). Add the solution at the bottom of each well without touching the side wall.
8. Seal the plate with a cover and incubate at 37° for 45 min.
9. Remove the cover, and wash plate 3 times with Wash buffer (Kit Component 10) using one of the following methods:
Manual Washing: Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with Wash buffer (Kit Component 10) and vortex mildly on ELISA shaker for 2 min, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure two more times for a **total of THREE washes.**
Automated Washing: Aspirate all wells, then wash plate **THREE times** with Wash buffer (Kit Component 10) (overfilling wells with the buffer). After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 min or shaking.
10. Add 0.1 ml of ABC working solution into each well, cover the plate and incubate at 37°C for 30 min.
11. Remove the cover and wash plate 5 times with Wash buffer (Kit Component 10), and each time let the wash buffer stay in the wells for 1-2 min. (See Step 9 for plate wash method).
12. Add 0.1 ml of TMB substrate (Kit Component 8) into each well, cover the plate and incubate at 37°C in dark within 30 min. (**Note:** This incubation time is for reference use only, the optimal time should be determined by end user.) And the shades of blue can be seen in the first 3-4 wells (with most concentrated Human Fetuin A standard solutions), the other wells show no obvious color.
13. Add 0.1 ml of Stop solution (Kit Component 9) into each well and mix thoroughly. The color changes into yellow immediately.
14. Read the O.D. absorbance at 450 nm in a microplate reader within 30 min after adding the stop solution.

5.3 Results

For calculation, $(\text{the relative O.D.}_{450}) = (\text{the O.D.}_{450} \text{ of each well}) - (\text{the O.D.}_{450} \text{ of Zero well})$. 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 human Fetuin A concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

6 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. It is recommended to use controls according to state and federal regulations. The use of controls is advised to assure the day to day validity of results. It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results. Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials, results of unknowns should be considered invalid.

In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. After checking the above mentioned items without finding any error contact your distributor or IBL-America directly.

7 PERFORMANCE CHARACTERISTICS

7.1 Range

780 pg/ml - 50,000 pg/ml

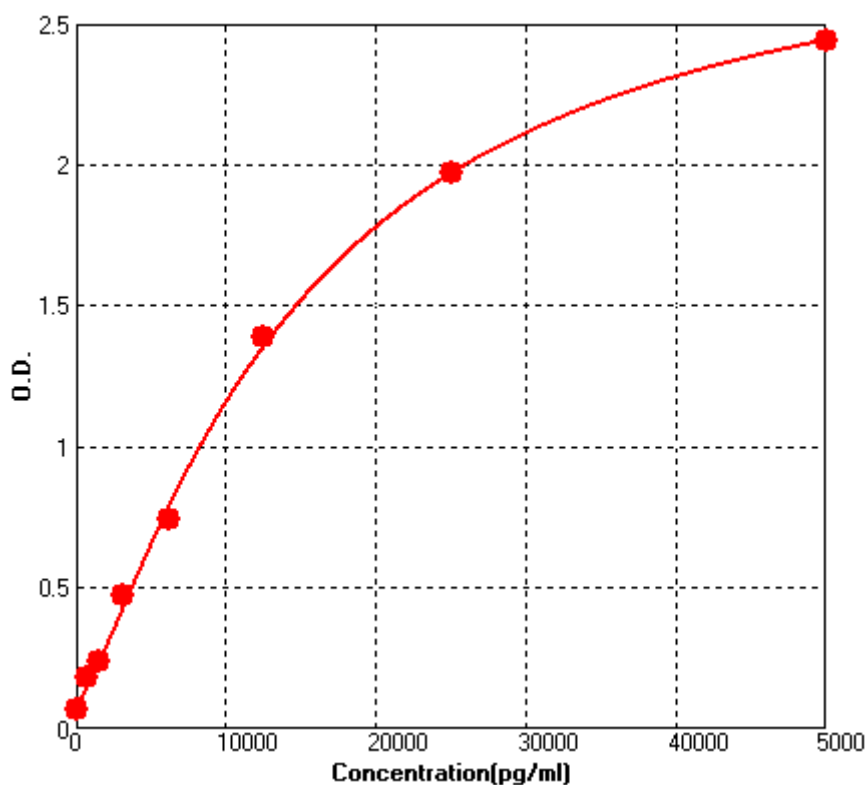
7.2 Sensitivity

<10pg/ml

7.3 Typical Data & Standard Curve

Results of a typical standard run of a human Fetuin A ELISA Kit are shown below. **This standard curve was generated at our lab for demonstration purpose only.** Each user should obtain their own standard curve as per experiment. (N/A=not applicable)

| X | pg/ml | 0 | 780 | 1560 | 3120 | 6250 | 12500 | 25000 | 50,000 |
|---|-------|-------|-------|-------|-------|-------|-------|-------|--------|
| Y | OD450 | 0.067 | 0.182 | 0.236 | 0.471 | 0.744 | 1.391 | 1.971 | 2.440 |



7.4 References:

1. Rizzu P, Baldini A (1995). "Three members of the human cystatin gene superfamily, AHSG, HRG, and KNG, map within one megabase of genomic DNA at 3q27". *Cytogenet. Cell Genet.* 70 (1-2): 26-8.
2. Osawa, M., Umetsu, K., Sato, M., Ohki, T., Yukawa, N., Suzuki, T., Takeichi, S. Structure of the gene encoding human alpha 2-HS glycoprotein (AHSG). *Gene* 196: 121-125, 1997.

8 ORDERING INFORMATION

This kit is manufactured for Immuno-Biological Laboratories, Inc. (IBL-America). For ordering information, please contact:

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