HUMAN FETUIN A/ α2 HS-GLYCOPROTEIN (AHSG) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN FETUIN A CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM, AND PLASMA

HUMAN FETUIN A KIT CAN BE CONTAMINATED. TAKE PRECAUTIONARY MEASURES TO PREVENT CONTAMINATION OF KIT REAGENTS WHILE RUNNING THIS ASSAY (i.e., WEAR MASK AND WASH HANDS PRIOR TO STARTING ASSAY).

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PURCHASE INFORMATION:

ELISA NAME	HUMAN FETUIN A ELISA
Catalog No.	SK00173-06
Lot No.	
Formulation	96 T
Standard Range	31.25-2000 pg/mL
Sensitivity	15 pg/mL
Sample Volume	100 µl
Sample Type	Serum, EDTA Plasma, Cell Culture
Specificity	Human Fetuin A
Dilution Factor	200,000 ***(Optimal dilutions should be determined by each laboratory for each application)***
Intra-assay Precision	6-8%
Inter-assay Precision	8-10%
Storage	2°C - 8°C

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INTRODUCTION

Human FETUIN A immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human FETUIN A in cell culture supernates, serum, and plasma. It contains recombinant human FETUIN A and antibodies raised against this protein. It has been shown to accurately quantify recombinant human FETUIN A. Results obtained with naturally occurring FETUIN A samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural human FETUIN A.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for FETUIN A has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any FETUIN A present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated polyclonal antibody specific for FETUIN A is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of FETUIN A bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ The kit should not be used beyond the expiration date on the kit label.

_ Do not mix or substitute reagents with those from other lots or sources.

_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

_ If samples generate values higher than the highest standard, dilute the samples with the Dilution Buffer and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
Fetuin A Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against human FETUIN A.	**173-06-01**	**1 plate**
FETUIN A Standard – 2000 pg/vial of recombinant human FETUIN A in a buffered protein base with preservatives; lyophilized.	**173-06-02**	**1 vial**
Detection Antibody Concentrate – 105 µL/vial, 100-fold concentrated of biotinylated polyclonal antibody against human FETUIN A with preservatives; lyophilized.	**173-06-03**	**1 vial**
Positive Control – one vial of recombinant human FETUIN A, lyophilized	**173-06-04**	**1 vial**
Streptavidin-HRP Conjugate - 60 µL/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP	**SAHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered protein based solution with preservatives	**DB01**	**2 bottles**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 botle**
TMB Substrate Solution - 11 mL of TMB substrate solution	**TMB01**	**1 bottle**
Stop Solution – 11 mL of 0.5M HCl	**S-STOP**	**1 bottle**
Plate sealer	**EAPS**	**1 piece**

STORAGE

Unopened Kit: Store at 2 – 8 °C for up to 6 months. For longer storage, unopened Standard, Positive Control and Detection Antibody should be stored at -20 or -70 °C. Do not use kit past expiration date.
Opened / Reconstituted Detection Antibody: Reconstituted standard and detection antibody could be stored for up to one month at -70°C. Diluted standard working solution and positive control should be prepared and used immediately. Diluted standard solution CAN NOT BE REUSED. Streptavidin-HRP Conjugate 200-fold concentrate and other components may be stored at 2 - 8°C for up to 6 months.
Microplate Wells: Return unused wells to the plastic bag containing the desiccant pack, reseal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted Hydrochloric acid. Appropriate care should be taken while handling this solution. We therefore recommend that this product be handled by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

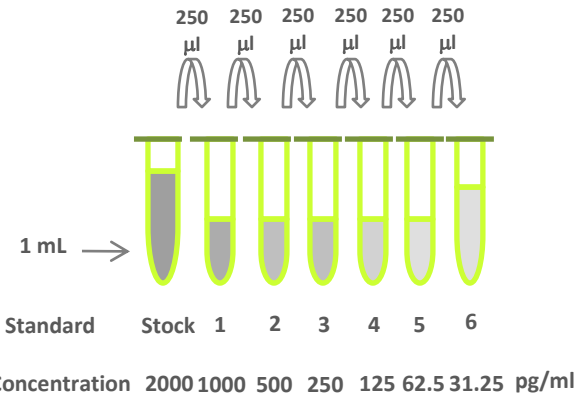
Serum and plasma samples require a 200000-fold dilution. A suggested 200000-fold dilution is 5 µL sample + 495 µL Dilution Buffer, following 5 µL 100-fold diluted sample solution + 495 µL Dilution Buffer, following 20 µL 10000-fold diluted sample solution + 380 µL Dilution Buffer. **Optimal dilutions should be determined by each laboratory for each application. Use polypropylene test tubes.**

REAGENT PREPARATION

Bring all reagents to room temperature before use.
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.

FETUIN A Standard - Refer to vial label for reconstitution volume. Reconstitute the **FETUIN A** Standard with 1 ml of Dilution Buffer. This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250µL of the Dilution Buffer into tubes #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

STANDARD	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	Powder	1000 µl	2000 pg/ml
# 1	250 µl of stock	250 µl	1000 pg/ml
# 2	250 µl of 1	250 µl	500 pg/ml
# 3	250 µl of 2	250 µl	250 pg/ml
# 4	250 µl of 3	250 µl	125 pg/ml
# 5	250 µl of 4	250 µl	62.5 pg/ml
# 6	250 µl of 5	250 µl	31.25 pg/ml



Detection Antibody - Reconstitute the **Detection Antibody Concentrate** with 105 µL of Dilution Buffer to produce a 100-fold concentrated stock solution. Pipette 10.395 mL of the Dilution Buffer into a 15 mL centrifuge tube and transfer 105 µL of 100-fold concentrated stock solution to prepare working solution.

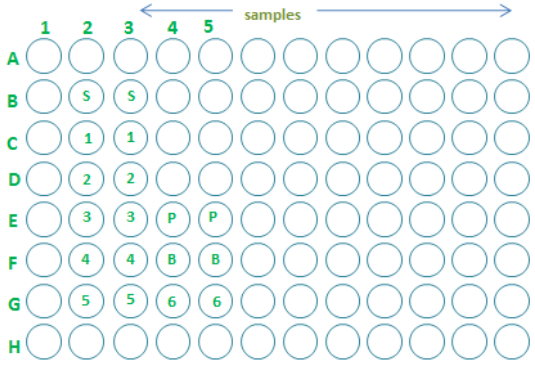
Streptavidin-HRP Conjugate - Pipette 11.94 mL of Dilution Buffer into the 15 ml centrifuge tube and transfer 60 µl of 200-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days.

Positive Control - Reconstitute the **Positive Control** with 1.0 mL of Dilution Buffer. **Note:** positive control should be prepared and used immediately.

ASSAY PROCEDURE

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

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch containing the desiccant pack, reseal.
3. Add 100 µL of Dilution Buffer to Blank well (F4, F5).
4. Add 100 µL of **Standard** (from B2, B3 to G2, G3 and G4, G5), **sample**, or **positive control** (E4, E5) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with **Wash Buffer** (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of **Detection Antibody working solution** to each well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 60 minutes on micro-plate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of **Substrate Solution** to each well. Incubate for 6-10 minutes at room temperature. **Protect from light.**
11. Add 100 µL of **Stop Solution** to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log 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 the graph. The data may be linearized by plotting the log of the FETUIN A concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Calculation of samples with a concentration exceeding that of standard 2000 pg/ml may result in inaccurate, low human Fetuin A levels. Such samples require further external pre-dilution according to expected human Fetuin A values with Dilution Buffer in order to precisely quantify the actual human Fetuin A level.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Fetuin A (pg/mL)	Average OD450 (Corrected)
Blank	0 (0.139)
31.25	0.014
62.5	0.036
125	0.090
250	0.199
500	0.471
1000	1.159
2000	2.607

- *Lot No.:
- ** Positive Control: 150 - 350 pg/mL

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human FETUIN A.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of FETUIN A was 15 pg/mL.

SPECIFICITY

This assay recognizes both natural and recombinant human FETUIN A. The factors listed below were prepared at 50 ng/mL in Dilution Buffer, and assayed for cross reactivity. Preparations of the following factors at 50 ng/mL in a mid-range rh FETUIN A control were assayed for interference. No significant cross-reactivity or interference was observed.

PROTEINS	CROSS-REACTIVITY
Human Fetuin A	100%
Mouse Fetuin A	0
Human Fetuin B	0
Human TGF-beta 1	0
Human BMP-2	0
Human MMP-9	0
Human Periostin	0
Human CRP	0
Human OPG	0
Human SPARC	0
Human FGF-23 N-Terminal	0

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5: Kanbay M, et al. Fibroblast Growth Factor 23 and Fetuin A are Independent Predictors for the Coronary Artery Disease Extent in Mild Chronic Kidney Disease. Clin J Am Soc Nephrol. 2010 Jun 24. [Epub ahead of print]

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100µl of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin-HRP Conjugate working solution to each well. Incubate 60 mins on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate solution to each well. Incubate 6-10 mins on the bench top**. Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min