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 EASILY. TAKE PRECAUTIONARY MEASURES TO PREVENT CONTAMINATION OF KIT REAGENTS WHILE RUNNING THIS ASSAY (i.e., WEAR MASK AND WASH HANDS PRIOR TO STARTING ASSAY).

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

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 - 4000 pg/mL
Sensitivity	15 pg/mL
Sample Volume	100 µL
Sample Type	Serum, EDTA Plasma, Cell Culture Supernates
Specificity	Human Fetuin A
Calibration	Human Fetuin A recombinant
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 – 8° C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human Fetuin A ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human Fetuin A in cell culture supernates, serum, and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human Fetuin A and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural Fetuin A samples.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human Fetuin A. The capture antibody can bind to the human Fetuin A in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human Fetuin A is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human Fetuin A bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

LIMITATIONS OF THE PROCEDURE

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

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
Fetuin A Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human Fetuin A.	**173-06-01**	**1 plate**
Fetuin A Standard – refer to refer to package label of recombinant human Fetuin A in a buffered protein base with preservative; lyophilized.	**173-06-02**	**vial**
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrate of biotinylated antibody against human Fetuin A with preservative; 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 preservative.	**DB01**	**2 bottles**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
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**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 8 months. For longer storage, unopened Standard, Positive Control and Detection Antibody should be stored at -20° C or -70° C. Do not use kit past expiration date.
Opened / Reconstituted Components: Reconstituted standard (stock) and Detection Antibody concentrated solution could be stored for up to one month at -70° C. Streptavidin-HRP Conjugate 200-fold concentrated solution (protect

from light) and other components may be stored at 2 – 8° C for up to 8 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8° C after opening.

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

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.
Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Serum and plasma samples require a 200,000-fold dilution. A suggested 100-fold dilution is 5 µL sample + 495 µL Dilution Buffer. Then, to make a 10,000-fold dilution is 5 µL of 100-fold diluted sample + 495 µL Dilution Buffer. Finally to make a 200,000-fold dilution is 20 µL 10,000-fold diluted sample + 380 µL Dilution Buffer. **Optimal dilutions should be determined by each laboratory for each application. Use polypropylene test tubes.**

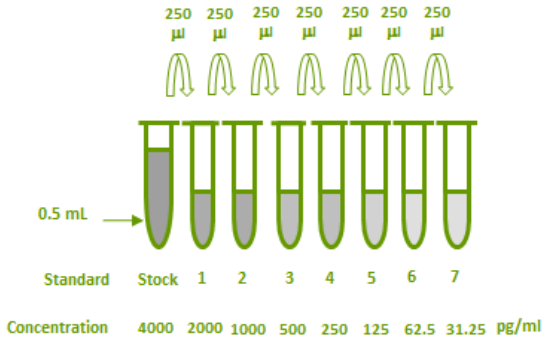
REAGENT PREPARATION

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 1x Wash Buffer.

Fetuin A Standard - Reconstitute the Fetuin A standard with refer to package label of Dilution Buffer. This reconstitution produces a stock solution of 4000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **4000 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	Powder	500 µl	4000 pg/ml
# 1	250 µl of stock	250 µl	2000 pg/ml
# 2	250 µl of 1	250 µl	1000 pg/ml
# 3	250 µl of 2	250 µl	500 pg/ml
# 4	250 µl of 3	250 µl	250 pg/ml
# 5	250 µl of 4	250 µl	125 pg/ml
# 6	250 µl of 5	250 µl	62.5 pg/ml
# 7	250 µl of 6	250 µl	31.25 pg/ml



Positive Control - Reconstitute the Positive Control with refer to package label of Dilution Buffer. **Note:** Positive Control could be reused in a few days if stored at -20° C or -70° C.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.05 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of the Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.94 mL of Dilution Buffer into a 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 (protect from light).

ELISA PROTOCOL

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

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 with the desiccant pack.
3. Add 100 µL of **Dilution Buffer** to Blank wells.
4. Add 100 µL of **standard dilutions** in reverse order of serial dilution, **samples**, or **positive control** per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with **1x 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 25-35 minutes on micro-plate shaker 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 4000 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 (0.081)
31.25	0.004
62.5	0.014
125	0.030
250	0.064
500	0.135
1000	0.391
2000	0.896
4000	1.744

SPECIFICITY

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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SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
⇩
Add 100 µl of standard dilutions, samples, or positive control to the 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 minutes on the plate shaker at RT. **Protect from light**.
⇩
Aspirate and wash 4 times.
⇩
Add 100 µl Substrate solution to each well. Incubate 25-35 minutes on the plate shaker at RT. **Protect from light**.
⇩
Add 100 µl Stop Solution to each well. Read 450nm within 15 min.