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

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

PURCHASE INFORMATION:

ELISA NAME	MOUSE FETUIN A ELISA
Catalog No.	SK00173-03
Lot No.	
Formulation	96 T
Standard Range	0.938-60 ng/mL
Sensitivity	0.47 ng/mL
Sample Volume	100 µl
Sample Type	Serum, EDTA Plasma, Cell Culture Supernates
Specificity	Mouse Fetuin A
Dilution Factor	2000 **(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

Mouse FETUIN A immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure mouse FETUIN A in cell culture supernates, serum, and EDTA plasma. It contains recombinant mouse FETUIN A and antibodies raised against this protein. It has been shown to accurately quantify recombinant mouse 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 mouse 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 monoclonal 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 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 monoclonal antibody against FETUIN A.	**173-03-01**	**1 plate**
FETUIN A Standard – 60 ng/vial of recombinant mouse FETUIN A in a buffered protein base with preservatives; lyophilized.	**173-03-02**	**1 vial**
Detection Antibody Concentrate – 105 µL/vial, 100-fold Concentrate of biotinylated monoclonal antibody against FETUIN A with preservatives; lyophilized.	**173-03-03**	**1 vial**
Positive Control – one vial of recombinant mouse FETUIN A, lyophilized	**173-03-04**	**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 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**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 6 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20 or -70 °C. Do not use kit past expiration date.

Opened / Reconstituted Components: Reconstituted standard could be stored for up to two weeks at -70° C. Diluted standard working

solution and positive control should be prepared and used immediately. Diluted standard solution CANNOT 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, therefore, should be taken while handling this solution.
We therefore recommend that this product is handled only 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.

Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

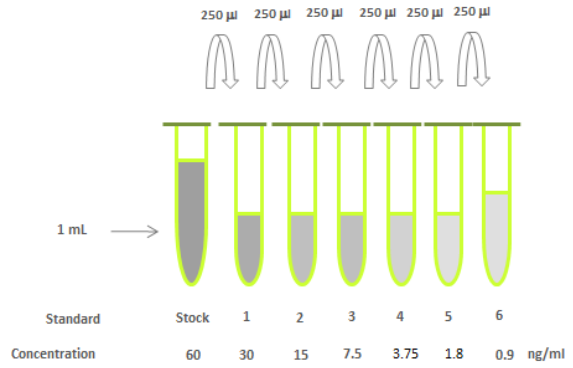
Serum and plasma samples may need a 2000-fold dilution. A suggested 2000-fold dilution is 10 µL sample + 990 µL Dilution Buffer to make a 100 fold sample solution, following 10 µL 100-fold diluted sample solution + 190 µL Dilution Buffer.
Optimal dilutions should be determined by each laboratory for 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 60 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250µL of 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 60 ng/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	Powder	1000 µl	60 ng/ml
# 1	250 µl of stock	250 µl	30 ng/ml
# 2	250 µl of 1	250 µl	15 ng/ml
# 3	250 µl of 2	250 µl	7.5 ng/ml
# 4	250 µl of 3	250 µl	3.75 ng/ml
# 5	250 µl of 4	250 µl	1.875 ng/ml
# 6	250 µl of 5	250 µl	0.938 ng/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 Dilution Buffer into a 15 ml centrifuge tube and transfer 105 µl of 100-fold concentrated stock solution to prepare working solution.

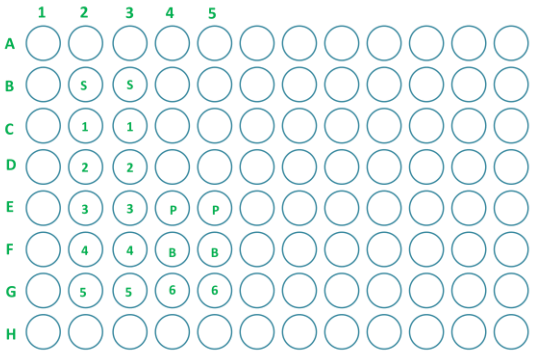
Streptavidin-HRP Conjugate - Pipette 11.94 mL of Dilution Buffer into a 15 ml centrifuge tube and transfer 60 µl of 200-fold concentrated stock solution to prepare working solution. **Note**: 1x working solution of Streptavidin HRP Conjugate should be used within a few days.

Positive Control - Reconstitute the **Positive Control** with 1 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 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 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 to 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 to 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 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 1 hour 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 8-12 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 60 ng/ml may result in inaccurate, low mouse Fetuin A levels. Such samples require further external predilution according to expected mouse Fetuin A values with Dilution Buffer in order to precisely quantitate the actual mouse Fetuin A level. Mouse serum or EDTA plasma may require 2000 fold dilution.

TYPICAL DATA

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

FETUIN A (NG/ML)	AVERAGE OD450 (CORRECTED)*
Blank	0 (0.183)
0.938	0.013
1.875	0.038
3.75	0.082
7.5	0.132
15	0.271
30	0.657
60	1.417

- *Lot No.:
- ** Positive Control: 3.5 – 7 ng/ml

CALIBRATION

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

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of FETUIN A was 0.47 ng/mL.

LINEARITY

To assess the linearity of the assay, pooled mouse EDTA plasma samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
1000X	124.731	124731	100
2000X	56.998	113996	91.4

To assess the linearity of the assay, pooled mouse serum samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
1000X	112.036	112036	100
2000X	58.051	116102	103.6

SPECIFICITY

This assay recognizes both natural and recombinant mouse FETUIN A. The factors listed below were prepared at 600 ng/mL in Dilution Buffer, and assayed for cross reactivity.

PROTEINS	CROSS-REACTIVITY
Mouse Fetuin A	100%
Human Fetuin A	0
Mouse FGF-23	0
Mouse BMP-4	0
Mouse MMP-9	0
Mouse MMP-2	0
Mouse OPG	0
Mouse CRP	0
Mouse FGF-21	0
Mouse Adiponectin	0

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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 1 hour on the plate shaker at RT. **Protect from light.**
⇩
Aspirate and wash 4 times.
⇩
Add 100 µl Substrate Solution to each well. Incubate 8-12 min on the bench top. **Protect from light.**
⇩
Add 100 µl Stop Solution to each well. Read 450nm within 15 min