MOUSE FETUIN A ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF MOUSE FETUIN A CONCENTRATIONS IN SERUM AND PLASMA

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.

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	MOUSE FETUIN A ELISA KIT
Catalog No.	SK00173-04
Lot No.	
Formulation	96 T
Standard Range	0.469- 60 ng/mL
Sensitivity	0.1 ng/mL
Sample Volume	100 µl
Sample Type	Serum, EDTA Plasma
Dilution factor	**10k ~ 40k (Optimal dilutions should be determined by each laboratory for each application)**
Specificity	Mouse Fetuin A
Calibration	Mouse Fetuin A Recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 – 8° C for 1 month. See page 2 for detail.
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

Order Contact:
AVISCERA BIOSCIENCE, INC.
2348 Walsh Ave., Suite C
Santa Clara, CA 95051
USA
Tel: (408) 982 0300
Fax: (408) 982 0301
Email: Sales@AvisceraBioscience.com
Info@AvisceraBioscience.com
www.AvisceraBioscience.com
www.AvisceraBioscience.net

DESCRIPTION

This Mouse Fetuin A ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural Mouse Fetuin A from serum and plasma in a sandwich ELISA format.

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

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for Mouse Fetuin A. The capture antibody can bind to the Mouse Fetuin A in the standard and samples. After washing the plate of any unbound substances, a monoclonal antibody HRP conjugate against Mouse Fetuin A is added to the wells. 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 Mouse 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.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix 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.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore 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 antibody against Fetuin A.	173-04-01	1 plate
Fetuin A Standard – refer to lot of recombinant Mouse FETUIN A in a buffered protein base with preservative; lyophilized.	173-04-02	1 vial
Detection Antibody HRP Concentrate – 105 µL/vial, 100-fold concentrate of antibody HRP conjugate against mouse Fetuin A with preservative; lyophilized.	173-04-03	1 vial
Positive Control - one vial of recombinant Mouse FETUIN A; lyophilized.	173-04-04	1 vial
Dilution Buffer – 40 mL of buffered protein based solution with preservative.	DB03	1 bottle
Antibody -HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	DB10B	1 bottle
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
Plastic Pouch	P01	1

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 1 month. For longer storage for up to 10 months, unopened Standard, Positive Control, Detection Antibody HRP Concentrate, Dilution Buffer and Antibody-HRP Diluent Solution should be stored at -20° C or -70° C.

TMB substrate should be stored only at 2-8 ˚C. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

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 may need an 10,000-40,000 fold dilution. A 100-fold dilution is 5 µL sample + 495 µL 1x Dilution Buffer. Finally, to make a 10,000-fold dilution is 5 µL of 100-fold sample + 495 µL 1x Dilution Buffer. Finally, to make an 20,000-fold dilution is 125 µL of 10,000-fold sample + 125 µL 1x Dilution Buffer. Finally, to make an 40,000-fold dilution is 60 µL of 10,000-fold sample + 180 µL 1x Dilution Buffer.

Optimal dilutions should be determined by each laboratory for each application.

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 lot 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. 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 BUFER	CONCENTRATION
Stock	Powder	Refer to lot	60 ng/ml
# 1	150 µl of stock	450 µl	15 ng/ml
# 2	150 µl of 1	450 µl	3.75 ng/ml
# 3	150 µl of 2	450 µl	0.978 ng/ml
# 4	250 µl of 3	250 µl	0.469 ng/ml

Positive Control - Reconstitute the positive control with refer to lot of Dilution Buffer to make positive control solution.

Detection Antibody HRP Concentrate –

Pipette 10.88 mL of **Antibody HRP Diluent Solution (DB10B)** into a 15 mL centrifuge tube and transfer 105 µL of 100-fold concentrated stock solution to prepare working solution.

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.

2. Add 100 µL per well of Dilution Buffer to Blank wells.
3. 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 microplate shaker at room temperature.
4. 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 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.
5. Add 100 µL of Detection Antibody HRP working solution to each well. Cover with plate sealer. Incubate for 90 minutes on microplate shaker at room temperature. **Protect from light.**
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of Substrate Solution to each well. Incubate for refer to lot at room temperature on microplate shaker. **Protect from light.**
10. 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.
11. Determine the optical density of each well using a microplate reader set to 450 nm within 3 minutes.

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.

TYPICAL DATA

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

STANDARD (NG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (refer to lot)
0.469	0.045
0.978	0.095
3.75	0.317
15	1.053
60	3.336

SPECIFICITY

PROTEINS	CROSS-REACTIVITY
Mouse Fetuin A	100%
Human Fetuin A	0
Rat Fetuin A	0

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 HRP working solution to each well. Incubate 90 min on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate solution to each well. Incubate refer to lot on plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read at 450nm within 3 min.