Epitope Diagnostics, Inc.

EDI™ Human Fetuin-A ELISA Kit

Enzyme Linked ImmunoSorbent Assay (ELISA) for the measurement of Human Fetuin-A Levels in Serum or Tissue Extract

REF **KT-800** Σ **96** +2 +8 °C

For Research Use Only **Not for Use in Diagnostic Procedures**

INTENDED USE

This ELISA (enzyme-linked immunosorbent assay) kit is produced for the quantitative determination of human fetuin-A or alpha 2-HS glycoprotein levels in serum, plasma, tissue extract or other liquid samples.

SUMMARY OF PHYSIOLOGY

Fetuin-A is a 59 kDa glycoprotein that consists of two amino-terminal cystatin domains and a smaller carboxyl-terminal domain. Fetuin-A is synthesized by the liver and secreted into blood stream, where its concentration in adult mammals ranges from 0.5 – 1.5 g/L. Fetuin-A occurs in high serum concentration during fetal life and involves in protease inhibitory activities and development-associated regulation of calcium metabolism and osteogenesis. It accumulates in bones and teeth as a major fraction of noncollagenous bone proteins. Biologically, studies have demonstrated that fetuin is the major calcification inhibitor found in serum, namely interference with calcium salt precipitation. Recent study has indicated that fetuin-A level drops in uremic patients on hemodialysis in comparison to normal healthy controls. The low fetuin-A level may associates with a higher cardiovascular mortality in patients on dialysis.

ASSAY PRINCIPLE

This ELISA is designed, developed and produced for the quantitative measurement of human fetuin-A in test sample. The assay utilizes the two-site “sandwich” technique with two selected polyclonal antibodies that bind to different epitopes of human fetuin.

Assay standards, controls and prediluted patient samples containing human fetuin-A are added to microtiter wells of microplate that was coated with a high affinity polyclonal anti-human fetuin-A antibody. After the first incubation period, the antibody on the wall of microtiter well captures human fetuin-A in the sample and unbound protein in each microtiter well is washed away. Then a HRP-conjugated polyclonal anti-human fetuin-A antibody is added to each microtiter well and a “sandwich” of “capture antibody - human fetuin - HRP-conjugated tracer antibody” is formed. The unbound tracer antibody is removed in the subsequent washing step. HRP-conjugated tracer antibody bound to the well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the tracer antibody bound to the fetuin-A on the wall of the microtiter well is directly proportional to the amount of fetuin-A in the sample. A standard curve is generated by plotting the absorbance versus the respective human fetuin-A concentration for each standard on point to point or cubical scales. The concentration of human fetuin-A in test samples is determined directly from this standard curve.

REAGENTS: Preparation and Storage

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.

1. **Fetuin Antibody Coated Microplate (Cat. No. 30010)**
One microplate with 12 x 8 strips (96 wells total) coated with antibody to human fetuin. The plate is framed and sealed in a foil Ziploc bag with a desiccant. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

2. **Fetuin Tracer Antibody (Cat. No. 30009)**
One vial containing 0.6 mL concentrated horseradish peroxidase (HRP)-conjugated anti-human fetuin tracer antibody in a stabilized protein matrix. This reagent must be diluted with Tracer Antibody Diluent before use. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

3. **Tracer Antibody Diluent (Cat. No. 30653)**
One vial containing 12 mL ready-to-use buffer. It should be only used for tracer antibody dilution according to the assay procedures. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

4. **Fetuin Assay Buffer Concentrate (Cat. No. 10011)**
One vial containing 11 mL of concentrated phosphate buffered saline based assay buffer with bovine serum albumin added. This concentrated assay buffer must be diluted 1:10 with distilled or deionized water (11 mL concentrate plus 99 mL distilled water) before use. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

5. **ELISA Wash Concentrate (Cat. No. 10010)**
One bottle contains 30 mL of 30-fold concentrate. Before use the contents must be diluted with 870 mL of distilled water and mix well. Upon dilution this yields a working wash solution containing a surfactant in phosphate buffered saline with a non-azide preservative. The diluted wash solution should be stored at room temperature and is stable until the expiration date on the kit box.

6. **ELISA HRP Substrate (Cat. No. 10020)**
One bottle contains 12 mL of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

7. **ELISA Stop Solution (Cat. No. 10030)**
One bottle contains 12 mL of 0.5 M sulfuric acid. This reagent should be stored at 2 – 8°C or room temperature and is stable until the expiration date on the kit box.

8. Fetuin Standards (Cat. No. 30001 – 30005)
Five vials each containing 0.5mL of human fetuin in a liquid bovine serum-based matrix with a non-azide preservative. **Refer to vials for exact concentration for each standard.** All the standards should be stored at -20°C or below after the first use with up to 3 freeze cycles.

9. Fetuin Controls (Cat. No. 30007 – 30008)
Two vials each containing 0.5mL of human fetuin in a liquid bovine serum-based matrix with a non-azide preservative. **Refer to vials for exact concentration range for each control.** Both controls should be stored at -20°C or below after the first use with up to 3 freeze cycles.

SAFETY PRECAUTIONS

The reagents must be used in a research laboratory and are for research use only. The source material for reagents containing bovine serum was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they are potentially infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 10 µL, 25 µL, 100 µL, and 1000 µL.
2. Repeating dispenser suitable for delivering 100 µL.
3. Disposable pipette tips suitable for above volume dispensing.
4. Disposable 12 x 75 mm or 13 x 100 glass tubes.
5. Disposable plastic 100 mL and 1000 mL bottle with caps.
6. Aluminum foil.
7. Deionized or distilled water.
8. Plastic microtiter well cover or polyethylene film.
9. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
10. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

SPECIMEN COLLECTION

Only 10 µL of human serum is required for human fetuin measurement. No special preparation of individual is necessary prior to specimen collection. Whole blood should be collected and must be allowed to clot for minimum 30 minutes at room temperature before the serum is separated by centrifugation (850 – 1500xg for 10 minutes). The serum should be separated from the clot within three hours of blood collection and transferred to a clean test tube. Serum samples should be stored at -20^0C or below until measurement. Avoid more than three freeze-thaw cycles of specimen.

ASSAY PROCEDURE

1. Patient Sample Preparation
Patient sample need to be diluted 1:10,000 with diluted assay buffer before being measured.

(1) Label 2 test tubes (12x75 mm) with 1A and 1B.
(2) Add 1 mL of assay buffer to each tube (both 1A and 1B).
(3) Pipet 10 µL of patient sample to tube 1A and mix well (1:100 dilution).
(4) Pipet 10 µL of diluted patient sample from 1A to tube 1B mix well (1:10,000 dilution).

2. Reagent Preparation

(1) Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.
(2) Fetuin Assay Buffer Concentrate (Cat. 10011) and ELISA Wash Concentrate (Cat. 10010) must be diluted to working solution prior to use. Please see REAGENTS section for details.

3. Assay Procedure

(1) Place a sufficient number of antibody coated microwell strips (Cat. 30010) in a holder to run human fetuin standards, controls and unknown samples in duplicate.
(2) Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3
A	STD 1	STD 5	SAMPLE 1
B	STD 1	STD 5	SAMPLE 1
C	STD 2	STD 6	SAMPLE 2
D	STD 2	STD 6	SAMPLE 2
E	STD 3	C 1	SAMPLE 3
F	STD 3	C 1	SAMPLE 3
G	STD 4	C 2	
H	STD 4	C 2	

(3) Add 25 µL of standards, controls and 1:10,000 diluted patient samples into the designated microwell.
(4) Add 100 µL of assay buffer to each well.
(5) Mix gently and cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light.
(6) Incubate plate at room temperature for 2 hours.
(7) Prepare working Tracer Antibody Working Solution by 1:21 fold dilution of the Fetuin Tracer Antibody (Cat. 30009) with the Tracer Antibody Diluent (Cat. 30653). For each strip, it is required to mix 1 mL of Tracer Antibody Diluent with 50 µL of Fetuin Tracer Antibody in a clean test tube.
(8) Remove the aluminum foil and plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
(9) Add 100 µL of above diluted tracer antibody working solution to each of the wells.
(10) Cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light.
(11) Incubate plate at room temperature for 30 minutes.
(12) Remove the aluminum foil and plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
(13) Add 100 µL of ELISA HRP Substrate (Cat. 10020) into each of the wells.
(14) Cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light.
(15) Incubate plate at room temperature for 20 minutes.
(16) Remove the aluminum foil and plate sealer. Add 100 µL of ELISA Stop Solution (Cat. 10030) into each of the wells. Mix gently.
(17) Read the absorbance at 450 nm within 10 minutes in a microplate reader.

NOTE: to reduce the background, one can set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 595 nm, 620 nm or 630 nm.

PROCEDURAL NOTES

1. It is recommended that all standards, controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light-sensitive reagents in the original amber bottles.
3. Store any unused antibody-coated strips in the foil Ziploc bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. Avoid air bubbles in the microwell, which can result in lower binding efficiency and higher CV% of duplicate reading.
7. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.

INTERPRETATION OF RESULTS

1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the STD 1 (0 ng/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The standard curve is generated by the corrected absorbance of all standard levels on the ordinate against the standard concentration on the abscissa using point-to-point or log-log paper. Computer assisted data reduction programs may also be used for the calculation of results.

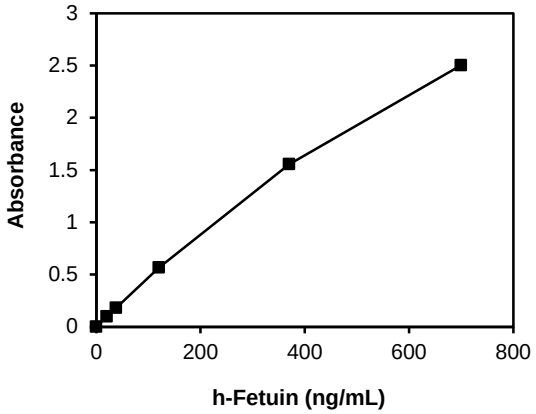
The human fetuin concentrations for the controls and 1:10,000 diluted samples are read directly from the standard curve using their respective corrected absorbance.

EXAMPLE DATA AND STANDARD CURVE

A typical absorbance data and the resulting standard curve from human fetuin ELISA are represented. **This curve should not be used in lieu of standard curve run with each assay.**

Well I.D.	OD 450 nm Absorbance			Results ng/mL
	Readings	Average	Corrected	
0 ng/mL	0.071 0.080	0.075	0.000	
20 ng/mL	0.174 0.173	0.174	0.099	
38 ng/mL	0.241 0.269	0.255	0.180	
120 ng/mL	0.629 0.653	0.641	0.566	
370 ng/mL	1.634 1.625	1.629	1.554	
700 ng/mL	2.564 2.590	2.577	2.502	
Control 1	0.327 0.379	0.353	0.348	58.8 ng/mL
Control 2	1.949 1.880	1.915	2.028	469.3 ng/mL

Human Fetuin ELISA



EXPECTED VALUES

Forty normal adult sera were measured with this human fetuin ELISA. The ninety-five percentile normal range was found to be 0.40 to 0.95 g/L with a mean value of 0.59 g/L and a standard deviation of 0.14 g/L.

The 10,000-fold dilution factor must be added to each sample for the original sample fetuin concentration. For example, a 1:10,000 diluted sample value is 24.3 ng/mL directly from the standard curve, the original sample fetuin concentration should be

24.3 ng/mL x 10,000 = 243000 ng/mL = 0.243 g/L

LIMITATION OF THE PROCEDURE

1. The lowest concentration of human fetuin directly measurable is 5 ng/mL, which represents serum fetuin concentration of 0.05 g/L to an original patient serum sample.
2. Since there is no Gold Standard concentration available for human fetuin measurement, the values of assay standards were established by diluting a highly purified recombinant human fetuin in a protein matrix.
3. For unknown sample value read directly from the assay that is greater than 350 ng/mL, it is recommended to measure a further diluted sample for more accurate measurement.
4. If there is not a microplate reader in your laboratory being able to read beyond 2.0 at OD 450 nm, run an assay without the standard level 6 from the standard set.
5. Bacterial or fungal contamination of serum specimens or reagents, or cross-contamination between reagents may cause erroneous results.
6. Water deionized with polyester resins may inactive the horseradish peroxidase enzyme.

QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls with known fetuin levels. We recommend that all assays include the laboratory's own fetuin controls in addition to those provided with this kit.

PERFORMANCE CHARACTERISTICS

Sensitivity

The sensitivity of the human fetuin ELISA as determined by the 95% confidence limit on 20 duplicate determination of zero standard is 2.5 ng/mL.

Precision

The intra-assay precision is validated by measuring two samples in a single assay with 20-replicate determinations.

Mean Fetuin Value (ng/mL)	CV (%)
33.6	5.5
121.1	4.8

The inter-assay precision is validated by measuring two samples in duplicate in 12 individual assays.

Mean Fetuin Value (ng/mL)	CV (%)
32.4	6.8
123.7	5.7

Linearity

Two human serum samples were diluted with assay buffer and assayed. The results in the value of ng/mL are as follows:

#	DILUTION	OBSERVED VALUE	EXPECTED VALUE	RECOVERY %
1	1:10,000	21.9		
	1:20,000	11.9	11.0	108
	1:40,000	5.3	5.5	96
	1:80,000	2.9	2.7	107
	1:160,000	1.6	1.4	114
2	1:10,000	192		
	1:20,000	99.9	96	104
	1:40,000	45.2	48	94
	1:80,000	22.2	24	93
	1:160,000	13.4	12	112

Recovery

Two patient samples were spiked with various amounts of human fetuin and assayed. The results in the value of ng/mL are as follows:

#	Orig. Value	Amount Spiked	Observed Value	Expected Value	Recovery %
1	33.6	21	25.1	27.3	92
		63	44.4	48.3	92
		200	120.1	116.8	103
2	121.1	21	68.9	71.1	97
		63	88.6	92.1	96
		200	157.1	160.6	98

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

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TECHNICAL ASSISTANCE AND CUSTOMER SERVICE
For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678. www.epitopediagnostics.com

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