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# High Sensitive Human Fetuin-A ELISA Assay Kit

Catalog Number:

HFA31-K01 (1 x 96 wells)

*For Research Use Only. Not for use in diagnostic procedures.*

*v. 1.0*

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EAGLE BIOSCIENCES, INC.

20A Northwest Blvd., Suite 112, Nashua, NH 03063

Phone: 617-419-2019 Fax: 617-419-1110

WWW.EAGLEBIO.COM



## **INTENDED USE**

Eagle Biosciences High Sensitive Human Fetuin-A ELISA (enzyme-linked immunosorbent assay) Assay Kit is intended for the quantitative determination of human Fetuin-A, also known as alpha-2-HS glycoprotein (AHSG), in serum. The Eagle Biosciences High Sensitive Human Fetuin-A ELISA Assay kit is intended for research use only and not intended for diagnostic procedures.

## **ASSAY BACKGROUND**

Fetuin-A, also known as alpha-2-HS glycoprotein, 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-A is the major calcification inhibitor found in serum, where it interferes with calcium salt precipitation. A 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 be associated with a higher cardiovascular mortality in patients on dialysis.

## **PRINCIPLE OF THE ASSAY**

This Eagle Biosciences High Sensitive Human Fetuin-A ELISA Assay Kit is designed, developed and produced for the quantitative measurement of human Fetuin-A in serum samples. The assay utilizes the two-site “sandwich” technique with two selected goat anti-human Fetuin-A polyclonal antibodies that bind to different epitopes of human Fetuin-A.

Assay standards, controls and prediluted patient serum samples containing human Fetuin-A are added to microtiter wells of a microplate that was coated with a high affinity polyclonal goat 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 proteins in each microtiter well are washed away. Then a horseradish peroxidase (HRP)-conjugated polyclonal anti-human Fetuin-A antibody is added to each microtiter well and a “sandwich” of “capture antibody – human Fetuin-A – 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 at 2 – 8°C 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-A Antibody Coated Microplate**

One microplate with 12 x 8 strips (96 wells total) coated with antibody to human Fetuin-A. 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. HS Fetuin-A Tracer Antibody**

One vial containing 0.6 mL concentrated horseradish peroxidase (HRP)-conjugated anti-human Fetuin-A 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**

One vial containing 12 mL ready-to-use Trizma Hydrochloride based buffer as supplied. 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-A Assay Buffer Concentrate**

One vial containing 30 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 (30 mL concentrate plus 270 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**

One bottle containing 30 mL of 30-fold concentrate. Before use the contents must be diluted with 870 mL of distilled water and mixed 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**

One bottle containing 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. Fetuin-A Standards**

Six vials each containing 0.5mL of human Fetuin-A 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.

### **8. Fetuin-A Controls**

Two vials each containing 0.5mL of human Fetuin-A 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 store at -20°C or below after the first use with up to 3 freeze cycles.

## **SAFETY PRECAUTIONS**

The reagents must be used in clinical reference laboratory and is intended for in vitro diagnostic use by medical or laboratory professionals 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 or



hydrogen peroxide. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. 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**

- Precision single channel pipettes capable of delivering 10  $\mu$ L, 25  $\mu$ L, 100  $\mu$ L, and 1000  $\mu$ L.
- Repeating dispenser suitable for delivering 100  $\mu$ L.
- Disposable pipette tips suitable for above volume dispensing.
- Disposable 12 x 75 mm or 13 x 100 glass or plastic tubes.
- Disposable plastic 100 mL and 1000 mL bottle with caps.
- Aluminum foil.
- Deionized or distilled water.
- Plastic microtiter well cover or polyethylene film.
- ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
- Spectrophotometric microplate reader capable of reading absorbance at 650 nm.

### **SPECIMEN COLLECTION**

Only 10  $\mu$ L of human serum is required for human Fetuin-A 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 may be stored at  $-20^{\circ}\text{C}$  or below until measurement. Avoid more than three freeze-thaw cycles of specimen.

### **ASSAY PROCEDURE**

#### **1. Patient Sample Preparation**

Patient sample needs to be diluted 1:300 with assay buffer before being measured.

- (1) Label 1 test tube (12x75 mm).
- (2) Add 3 mL of assay buffer to tube.
- (3) Pipet 10  $\mu$ L of patient sample to tube and mix well (1:300 dilution).

*Note: It is recommended to use a precision/calibrated pipette and careful technique to perform the dilution in order to get precise results! We recommend using Eppendorf Repeat Pipette with 12.5 mL combitip for adding 3 mL assay buffer. 50 mL combitip is not recommended.*

#### **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-A Assay Buffer Concentrate and ELISA Wash Concentrate 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 in a holder to run human Fetuin-A 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 10  $\mu\text{L}$  of standards, controls and 1:300 diluted patient samples into the designated microwell.
- (4) Add 100  $\mu\text{L}$  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 the plate at room temperature, shaking at 170 rpm for **90** minutes.
- (7) Prepare Tracer Antibody Working Solution by 1:21 fold dilution of the Fetuin-A Tracer Antibody with the Tracer Antibody Diluent . For each strip, it is required to mix 1 mL of Tracer Antibody Diluent with 50  $\mu\text{L}$  of Fetuin-A Tracer Antibody in a clean test tube.
- (8) Remove the aluminum foil and the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350  $\mu\text{L}$  of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
- (9) Add 100  $\mu\text{L}$  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 the plate at room temperature, shaking at 170 rpm for **45** minutes.
- (12) Remove the aluminum foil and the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350  $\mu\text{L}$  of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
- (13) Add 100  $\mu\text{L}$  of ELISA HRP Substrate into each of the wells.
- (14) Cover the plate with aluminum foil to avoid exposure to light.
- (15) Incubate the plate at room temperature, shaking at 170 rpm for 30 minutes.
- (16) Immediately read the absorbance at **650 nm** in a microplate reader.

#### 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 as this could 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. Appropriate computer assisted data reduction programs may also be used for the calculation of results.

The human Fetuin-A concentrations for the controls and 1:300 diluted samples are read directly from the standard curve using their respective corrected absorbance.

The 300-fold dilution factor must be added to each sample for the original sample Fetuin-A concentration. For example, a 1:300-fold diluted sample value is 1670 ng/mL directly from the standard curve, the original sample Fetuin-A concentration should be

$$(1670 \text{ ng/mL} \times 300) / 1,000,000 = 0.50 \text{ g/L}$$

## **EXPECTED VALUES**

Forty normal adult sera were measured with this human Fetuin-A ELISA. The ninety-five percentile normal range was found to be 0.5 to 1.0 g/L with a mean value of 0.733 g/L and a standard deviation of 0.178 g/L.

Forty sera from ESRD (end stage renal disease) patients were also measured with this assay. Twenty eight (70%) of them were below normal cut off (0.5 g/L). The Fetuin-A level was ranging from 0.197 g/L to 0.925 g/L.

## **LIMITATION OF THE PROCEDURE**

1. Since there is no Gold Standard concentration available for human Fetuin-A measurement, the values of assay standards were established by diluting a highly purified recombinant human Fetuin-A in a protein matrix.
2. For unknown sample value read directly from the assay that is greater than 4343 ng/mL, it is recommended to measure a further diluted sample for more accurate measurement.
3. Bacterial or fungal contamination of serum specimens or reagents, or cross-contamination between reagents may cause erroneous results.
4. Water deionized with polyester resins may inactivate the horseradish peroxidase enzyme.

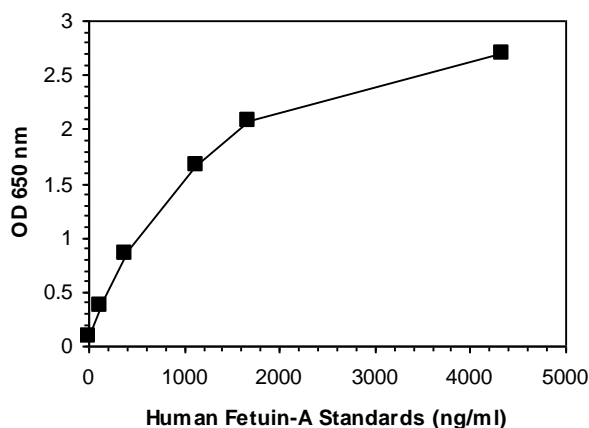


## EXAMPLE DATA AND STANDARD CURVE

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

Well I.D.	OD 650 nm Absorbance			Results ng/mL
	Readings	Average	Corrected	
0 ng/mL	0.091 0.092	0.092	0.000	
127 ng/mL	0.390 0.370	0.380	0.288	
376 ng/mL	0.865 0.840	0.852	0.760	
1133 ng/mL	1.623 1.714	1.668	1.576	
1670 ng/mL	2.095 2.067	2.081	1.989	
4343 ng/mL	2.576 2.832	2.704	2.612	
Control 1	1.165 1.117	1.141	1.049	644 ng/mL
Control 2	2.166 2.292	2.229	2.137	2305 ng/mL

### Human Fetuin-A ELISA



## QUALITY CONTROL

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

## PERFORMANCE CHARACTERISTICS

### Sensitivity

The analytical sensitivity of the Eagle Biosciences Human Fetuin-A ELISA Assay Kit as determined by measuring diluted samples of the lowest standard was 40 ng/mL.

### Precision



The intra-assay precision is validated by measuring two patient samples in a single assay with 15 replicate determinations.

Mean Fetuin-A Value (g/L)	CV (%)
0.392	1.6
0.457	2.2

The inter-assay precision is validated by measuring two samples in duplicate in 3 individual assays.

Mean Fetuin-A Value (g/L)	CV (%)
0.191	5.3
0.600	1.7

The reproducibility of different dilutions of patient sample was validated. Two patient samples were diluted in six separated 1:300 dilutions according to the assay procedure and all the diluted samples were measured in duplicate with this assay.

Mean Fetuin-A Value (g/L)	CV (%)
0.479	7.09
0.472	7.69

### Linearity

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

#	DILUTION	OBSERVED VALUE	EXPECTED VALUE	RECOVERY %
1	Original	1.303		
	1:2	0.733	0.652	112
	1:4	0.314	0.326	96
	1:8	0.163	0.163	100
	1:16	0.081	0.081	100
	1:32	0.040	0.041	98
2	Original	0.504		
	1:2	0.278	0.252	110
	1:4	0.130	0.126	103
	1:8	0.060	0.063	95
	1:16	0.030	0.032	94

### Recovery

Two patient samples were spiked with various amounts of human Fetuin-A and assayed. The results in the value of g/L are as follows:





#	Orig. Value (g/L)	Observed Value	Expected Value	Recovery %
1	0.732	0.278	0.268	104
		0.380	0.423	90
		0.472	0.577	82
2	0.425	0.320	0.361	89
		0.337	0.383	88
		0.383	0.404	95

## REFERENCES

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## Warranty Information

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at [info@eaglebio.com](mailto:info@eaglebio.com) or at 866-411-8023.