DRG® Fetuin A (Human) (EIA-4522)

Revised 28 Aug. 2013 rm (Vers. 3.1) **RUO in the USA**

This kit is intended for Research Use Only.

Not intended for use in diagnostic procedures.

Please use only the valid version of the package insert provided with the kit.

1 INTENDED USE

This ELISA (enzyme-linked immunosorbent assay) kit is intended for measurement of human Fetuin-A, also known as alpha-2-HS glycoprotein (AHSG), in serum, plasma, cell culture supernatant, tissue extraction and urine.

2 ASSAY PRINCIPLE

This ELISA is designed, developed and produced for 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 serum samples containing human Fetuin-A are added to microtiter wells of 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 protein in each microtiter well is 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.

3 REAGENTS: PREPARATION AND STORAGE

This test kit must be stored at 2 °C - 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 °C - 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 eight 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 °C - 8 °C and is stable until the expiration date on the kit box.

2. 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 °C - 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 °C - 8 °C and is stable until the expiration date on the kit box.

4. Fetuin-A Assay Buffer Concentrate
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 °C - 8 °C and is stable until the expiration date on the kit box.

5. ELISA Wash Concentrate
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 Solution
One bottle contains 12 mL of tetramethylbenzidine (TMB) with hydrogen peroxide.
This reagent should be stored at 2 °C - 8 °C and is stable until the expiration date on the kit box.

7. ELISA Stop Solution
One bottle contains 12 mL of 0.5 M sulfuric acid.
This reagent should be stored at 2 °C - 8 °C or room temperature and is stable until the expiration date on the kit box.

8. Fetuin-A Standards
Five vials each contain human Fetuin-A in a liquid bovine serum based matrix with a non-azide preservative.
Refer to vial 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-A Controls
Two vials each contain 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.

4 SAFETY PRECAUTIONS

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.

5 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 or plastic 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.

6 SPECIMEN COLLECTION

Only 10 µL of **human serum or plasma** 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 – 1500 x g 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 °C or below until measurement. Avoid more than three freeze-thaw cycles of specimen.

Twenty four hour urine sample is recommended to be used for the determination of urine Fetuin-A concentration.

Spot urine from the second morning urination may be used if strenuous physical activity shortly before sample collection has been ruled out and polyuric renal dysfunction is not present. Intra-individual day-to-day fluctuations in the concentration of urine proteins caused by diurisis may be reduced by relating to the urinary creatinine concentration.

For **cell culture supernatant, tissue extracts**, one should serial dilute test sample and measure multiple diluted samples for a more accurate Fetuin-A test result.

7 ASSAY PROCEDURE

7.1 Sample Preparation

Serum or plasma sample needs to be diluted 1:10,000 with 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 serum or plasma sample to tube 1A and mix well (1:100 dilution).

(4) Pipet 10 µL of diluted sample from 1A to tube 1B mix well (1:10,000 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 1 ml assay buffer, 50 mL combitip is not recommended.

Urine sample needs to be diluted 1:100 with assay buffer before being measured.

1. Label 1 test tube (12 x75 mm) with 1.
2. Add 1 mL of assay buffer to tube.
3. Pipet 10 µL of urine sample to tube 1 and mix well (1:100 dilution).

Note: If a higher than standard level 5 of Fetuin-A test result is obtained, a further dilution of urine sample (e.g. 1:500) should be measured for reporting a more accurate test result.

7.2 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.

7.3 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 2
B	STD 1	STD 5	SAMPLE 2
C	STD 2	C 1	SAMPLE 3
D	STD 2	C 1	SAMPLE 3
E	STD 3	C 2	
F	STD 3	C 2	
G	STD 4	SAMPLE 1	
H	STD 4	SAMPLE 1	

3. Add 25 µL of standards, controls and 1:10,000 diluted samples into the designated microwell.
   Note: if urine sample is used, 1:100 diluted urine sample should be used.
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 the plate at room temperature for 2 hours.
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 µL 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 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 the plate at room temperature for 30 minutes.
12. Remove the aluminum foil and the 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 into each of the wells.
14. Cover the plate with aluminum foil to avoid exposure to light.
15. Incubate the plate at room temperature for 20 minutes
16. Remove the aluminum foil. Add 100 µL of ELISA Stop Solution 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 or 620 nm or 630 nm.

8 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.

9 CALCULATING 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.
4. We recommend a linear ordinate for optical density and a linear abscissa for concentration.

The human serum or plasma Fetuin-A concentrations for the controls and 1:10,000 diluted samples are read directly from the standard curve using their respective corrected absorbance.

10 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.**

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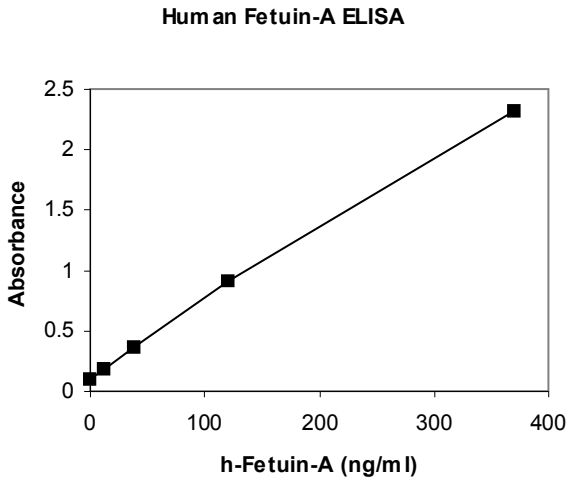
Well I.D.	OD 450 nm Absorbance			Results ng/mL
	Readings	Average	Corrected	
0 ng/mL	0.097 0.099	0.098	0.000	
12.5 ng/mL	0.173 0.185	0.179	0.081	
38 ng/mL	0.364 0.379	0.371	0.273	
120 ng/mL	0.876 0.949	0.913	0.815	
370 ng/mL	2.298 2.325	2.312	2.214	
Control 1	0.471 0.498	0.484	0.386	55.1 ng/mL
Control 2	1.697 1.700	1.690	1.592	258.9 ng/mL



The 10,000-fold dilution factor must be added to each sample for the original sample Fetuin-A concentration.

For example, a 1/10,000 fold diluted sample value is 24.3 ng/mL directly from the standard curve, the original sample Fetuin-A concentration should be

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

11 LIMITATION OF THE PROCEDURE

a. The lowest concentration of human Fetuin-A directly measurable is 5.0 ng/mL (assay analytical sensitivity). After back calculation for the 1/10,000 fold dilution of serum sample, the assay measures the lowest serum Fetuin-A concentration at 50 µg/mL of original serum sample.

b. 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.

c. 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.

d. If there is not a microplate reader in your laboratory being able to read beyond 2.0 at OD 450 nm, one can just run an assay without the standard level 6 from the standard set.

e. Bacterial or fungal contamination of serum specimens or reagents, or cross-contamination between reagents may cause erroneous results.

f. Water deionized with polyester resins may inactive the horseradish peroxidase enzyme.

12 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.

1 REFERENCES

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