# RAT FETUIN A ELISA KIT

**FOR THE QUANTITATIVE DETERMINATION OF RAT FETUIN A CONCENTRATIONS IN CELL CULTURE SUPERNATES**

**PURCHASE INFORMATION:**

| ELISA NAME | RAT FETUIN A ELISA |
|---|---|
| Catalog No. | SK00173-02 |
| Lot No. | |
| Formulation | 96 T |
| Standard Range | 15.6 – 2000 pg/mL |
| Sensitivity | 5 pg/mL |
| Sample Volume | 100 µL |
| Sample Type | Cell Culture Supernates |
| Specificity | Rat Fetuin A |
| Dilution Factor | Optimal dilutions should be determined by each laboratory for each application |
| Intra-assay Precision | 6-8% |
| Inter-assay Precision | 8-10% |
| Storage | 2-8°C |

**FOR RESEARCH USE ONLY.NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

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## INTRODUCTION

Rat Fetuin A immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure rat Fetuin A in cell culture supernates (other sample types need to be validated prior to assay). It contains recombinant rat Fetuin A and antibodies raised against this protein. It has been shown to accurately quantify recombinant rat Fetuin A. The immunoassay kit can be used to determine relative mass values for natural rat Fetuin A.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for rat 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 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

_ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_ 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 an antibody against Fetuin A. | **173-02-01** | **1 plate** |
| **Fetuin A Standard** – 2000 pg/vial of recombinant rat Fetuin A in a buffered protein base with preservatives; lyophilized. | **173-02-02** | **1 vial** |
| **Detection Antibody Concentrate** – 1.05 mL/vial, 10-fold concentrate of biotinylated antibody against Fetuin A with preservatives; lyophilized. | **173-02-03** | **1 vial** |
| **Positive Control** – one vial of recombinant rat Fetuin A; lyophilized | **173-02-04** | **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 | **DB08** | **1 bottle** |
| **HRP Diluent Solution** - 12 mL of buffered protein based solution with preservatives | **DB01** | **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 8 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 (stock) and Detection Antibody concentrated solution could be stored for

up to one month at -20 or -70 °C. Streptavidin-HRP Conjugate 200-fold concentrated solution (protect from light) and other components may be stored at 2 – 8 °C for up to 8 months.
**Microplate Wells:** Return unused wells to the plastic pouch with the desiccant pack and seal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 – 8 °C after opening.

## 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.
**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

**Optimal dilutions should be determined by each laboratory for each application.**
**Use polypropylene test tubes.**

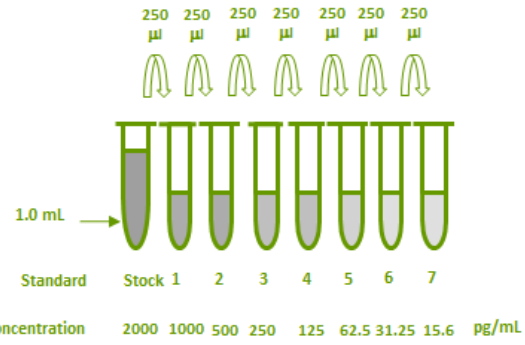
## REAGENT PREPARATION

**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 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

| TUBE | STANDARD | DILUTION BUFFER | CONCENTRATION |
|---|---|---|---|
| Stock | Powder | 1000 µl | 2000 pg/mL |
| # 1 | 250 µl of stock | 250 µl | 1000 pg/mL |
| # 2 | 250 µl of 1 | 250 µl | 500 pg/mL |
| # 3 | 250 µl of 2 | 250 µl | 250 pg/mL |
| # 4 | 250 µl of 3 | 250 µl | 125 pg/mL |
| # 5 | 250 µl of 4 | 250 µl | 62.5 pg/mL |
| # 6 | 250 µl of 5 | 250 µl | 31.25 pg/mL |
| # 7 | 250 µl of 6 | 250 µl | 15.6 pg/mL |




**Positive Control** - Reconstitute the **Positive Control** with 1.0 mL of Dilution Buffer. **Note**: Positive Control should be prepared and used immediately.

**Detection Antibody -** Reconstitute the **Detection Antibody Concentrate** with 1.05 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Dilution Buffer into a 15

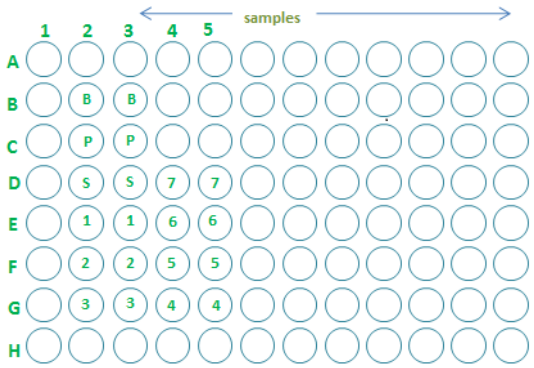
mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

**Streptavidin-HRP Conjugate** - Pipette 11.94 mL of **HRP Diluent Solution (DB01)** 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 (protect from light).

## ASSAY PROCEDURE

**Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples 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 with the desiccant pack and seal.
3. Add 100 µL of Dilution Buffer to Blank wells (B2, B3).
4. Add 100 µL of Standard (reverse order of serial dilution, from D4, D5 to G4, G5 and G2, G3 to D2, D3), sample, or positive control (C2, C3) 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 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.
6. Add 100 µL of Detection Antibody working solution to each well. Cover with plate 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 3-6 minutes on micro-plate shaker 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 samples, 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 2000 pg/mL may result in inaccurate, low rat Fetuin A levels. Such samples require further external predilution according to expected rat Fetuin A values with Dilution Buffer in order to precisely quantify the actual rat Fetuin A level.

## TYPICAL DATA

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

| FETUIN A (PG/ML) | AVERAGE OD450 (CORRECTED)* |
|---|---|
| **Blank** | 0 (0.053) |
| **15.625** | 0.027 |
| **31.25** | 0.055 |
| **62.5** | 0.126 |
| **125** | 0.201 |
| **250** | 0.397 |
| **500** | 0.913 |
| **1000** | 1.302 |
| **2000** | 2.255 |

- Lot No.: 20111021
- Positive Control: 100 – 300 pg/mL

## CALIBRATION

This immunoassay is calibrated against a highly purified recombinant rat Fetuin A.

## SENSITIVITY

5 pg/mL

## SPECIFICITY

This assay recognizes both natural and recombinant rat Fetuin A. The factors listed below were prepared at 50 ng/mL in Dilution Buffer, and assayed for cross reactivity.

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

## REFERENCES:

1: Voigt M, et al. Fibroblast growth factor (FGF)-23 and Fetuin-A in calcified carotid atheroma. Histopathology. 2010 May;56(6):775-88.

2: Ishibashi A, et al . Serum Fetuin-A is an Independent Marker of Insulin Resistance in Japanese Men. J Atheroscler Thromb. 2010 Jun 11. [Epub ahead of print]

3: Roos M, et al. Serum fetuin-A, cardiovascular risk factors, and six-year follow-up outcome in patients with coronary heart disease. Am J Cardiol. 2010 Jun 15;105(12):1666-72. Epub 2010 Apr 27.

4: Yuce M, et al. Fetuin-A, osteoporosis and inflammation--proposal of possible mechanisms for vascular and valvular calcification in chronic kidney disease. Nephrol Dial Transplant. 2010 May 24. [Epub ahead of print]

5: Kanbay M, et al. Fibroblast Growth Factor 23 and Fetuin A are Independent Predictors for the Coronary Artery Disease Extent in Mild Chronic Kidney Disease. Clin J Am Soc Nephrol. 2010 Jun 24. [Epub ahead of print]

## SUMMARY OF ASSAY PROCEDURE

| PREPARE REAGENTS, SAMPLES AND STANDARDS |
|---|
| ↓ |
| Add 100 µl 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 3-6 min on the plate shaker. **Protect from light.** |
| ↓ |
| Add 100 µl Stop Solution to each well. Read 450nm within 15 min |