

## Human Fetuin A ELISA kit

### Cat#: orb50196 (Product Manual)

#### Assay Principle

The Biorbyt Human AHSB Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Human AHSB with a 96-well strip plate that is pre-coated with antibody specific for AHSB. The detection antibody is a biotinylated antibody specific for AHSB. The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat. The kit contains recombinant Human AHSB with immunogen: Expression system for standard: NSO; Immunogen sequence: A19-V367. The kit is analytically validated with ready to use reagents.

To measure Human AHSB, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is substrate to HRP and will be catalyzed to produce a blue color product, which changes into yellow after adding acidic stop solution. The density of the yellow product is linearly proportional to Human AHSB in the sample. Read the density of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Human AHSB in the sample.

#### Overview

Product Name	Human Fetuin A / AHSB ELISA Kit
Reactive Species	Human
Size	96wells/kit, with removable strips.
Description	Sandwich High Sensitivity ELISA kit for Quantitative Detection of Human Fetuin A. 96wells/kit, with removable strips.
Sensitivity	<10pg/ml *The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.
Detection Range	0.78ng/ml-50ng/ml
Storage Instructions	Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles(Shipped with wet ice.)
Uniprot ID	P02765

## Technical Details

Capture/Detection Antibodies	The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat.
Specificity	Natural and recombinant Human AHSG
Immunogen	Expression system for standard: NSO; Immunogen sequence: A19-V367
Cross Reactivity	There is no detectable cross-reactivity with other relevant proteins.

## Notice Before Application

Please read the following instructions before starting the experiment.

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
3. Don't let 96-well plate dry, for dry plate will inactivate active components on plate.
4. Don't reuse tips and tubes to avoid cross contamination.
5. Avoid using the reagents from different batches together.

## Kit Components/Materials Provided

Description	Quantity	Volume	Storage of opened/reconstituted material
Anti-Human AHSG Pre-coated 96-well Strip Microplate	1	12 strips of 8 wells	Return unused wells to the foil pouch. Reseal along the entire edge of the zip-seal. May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit.
Human AHSG Standard	2	50 ng/tube	Discard the AHSG stock solution after 12 hours at 4°C. May be stored at -20°C for 48 hours.
Human AHSG Biotinylated Antibody (100x)	1	100 µl	May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit.
Avidin-Biotin-Peroxidase Complex (100x)	1	100 µl	
Sample Diluent	1	30 ml	
Antibody Diluent	1	12 ml	
Avidin-Biotin-Peroxidase Diluent	1	12 ml	
Color Developing Reagent (TMB)	1	10 ml	
Stop Solution	1	10 ml	
Wash Buffer (25x)	1	20 ml	
Plate Sealers	4	Piece	

Activate  
Go to Set

### Required Materials That Are Not Supplied

- Microplate Reader capable of reading absorbance at 450nm.
- Automated plate washer (optional)
- Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions. Multichannel pipettes are recommended for large amount of samples.
- Deionized or distilled water.
- 500ml graduated cylinders.
- Test tubes for dilution.
- Washing buffer (neutral PBS or TBS).

Preparation of 0.01M TBS: Add 1.2g Tris, 8.5g NaCl; 450µl of purified acetic acid or 700µl of concentrated hydrochloric acid to 1000ml H<sub>2</sub>O

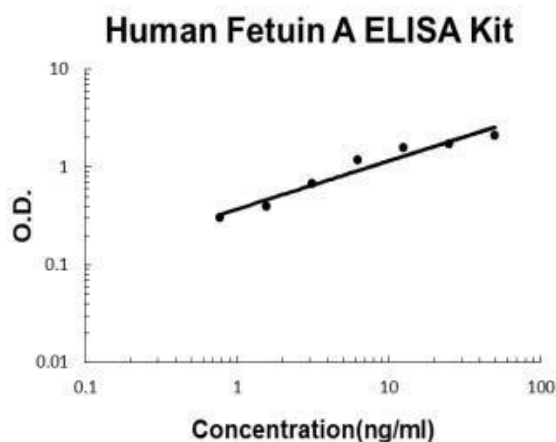
Preparation of 0.01 M PBS: Add 8.5g sodium chloride, 1.4g Na<sub>2</sub>O and adjust pH to 7.2-7.4

### Human Fetuin A / AHSR ELISA Kit Standard Curve Example

Highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

Concentration (ng/ml)		0.78	1.56	3.12	6.25	12.5	25	50
O.D.	0.071	0.306	0.396	0.673	1.169	1.585	1.719	2.126

Human Fetuin A ELISA Kit standard curve



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

### Intra/Inter Assay Variability

Biorbyt spend great efforts in documenting lot to lot variability and make sure our assay kits produce robust data that are reproducible.

**Intra-Assay Precision (Precision within an assay):** Three samples of known concentration were tested on one plate to assess intra-assay precision.

**Inter-Assay Precision (Precision across assays):** Three samples of known concentration were tested in separate assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean(pg/ml)	1646	4628	22095	1708	5021	21195
Standard deviation	126.74	185.82	883.8	143.47	2244	1229.31
CV(%)	7.7%	4%	4%	8.4%	4.5%	5.8%

### Reproducibility

To assay reproducibility, three samples with differing target protein concentrations were assayed using four different lots.

Lots	Lot1 (pg/ml)	Lot2 (pg/ml)	Lot3 (pg/ml)	Lot4 (pg/ml)	Mean (pg/ml)	Standard Deviation	CV (%)
Sample 1	1646	1726	1766	1528	1666	90.88	5.4%
Sample 2	4628	4495	4603	4388	4528	95.27	2.1%
Sample 3	22095	25102	22613	25109	23729	1387.88	5.8%

\*number of samples for each test n=16.

## Preparation Before The Experiment

Item	Preparation
All reagents	Bring all reagents to room temperature (18-25°C) prior to use. Please DO NOT equilibrate unused plate well strips to room temperature. They should be sealed and stored in the original packaging. The assay can also be done at room temperature however we recommend doing it at 37°C for best consistency with our QC results. Also, the TMB incubation time estimate (15-25 min) is based on incubation at 37°C.
Wash buffer	Prepare 500 ml of Working Wash Buffer by diluting the supplied 20 ml of Wash Buffer (25 x) with 480 ml of deionized or distilled water. If crystals have formed in the concentrate, warm to room temperature and mix it gently until crystals have completely dissolved.
Biotinylated Anti-Human AHSG antibody	It is recommended to prepare this reagent immediately prior to use by diluting the Human AHSG Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µl by adding 1 µl of Biotinylated antibody (100x) to 99 µl of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Avidin-Biotin-Peroxidase Complex	It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µl by adding 1 µl of Avidin-Biotin-Peroxidase Complex (100x) to 99 µl of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Human AHSG Standard	It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 50 ng of lyophilized Human AHSG standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 50 ng/ml using 1ml of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.

## Dilution of Human AHSG Standard

1. Number tubes 1-8. Final Concentrations to be Tube # 1 –50000pg/ml, #2 –25000pg/ml, #3 – 12500pg/ml, #4 – 6250pg/ml, #5 – 3125pg/ml, #6 – 1562.5pg/ml, #7 – 781.25pg/ml, #8 – Sample Diluent serves as the zero standard (0pg/ml).
2. For standard #1, add 1000µl of undiluted standard stock solution to tube #1.
3. Add 300 µl of sample diluent to tubes # 2-7.
4. To generate standard #2, add 300 µl of standard #1 from tube #1 to tube #2 for a final volume of 600 µl. Mix thoroughly.
5. To generate standard #3, add 300 µl of standard #2 from tube #2 to tube #3 for a final volume of 600 µl. Mix thoroughly.
6. Continue the serial dilution for tube #4-7.

## Sample Preparation and Storage

These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

Sample Type	Procedure
Cell culture supernatants	Clear sample of particulates by centrifugation, assay immediately or store samples at -20°C.
Serum	Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.
Plasma	Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. Assay immediately or store samples at -20°C. *Note: it is important to not use anticoagulants other than the ones described above to treat plasma for other anticoagulants could block the antibody binding site.
Cell lysates	Lyse the cells, make sure there are no visible cell sediments. Centrifuge cell lysates at approximately 10000 X g for 5 min. Collect the supernatant.

## Sample Dilution

The target protein concentration should be estimated and appropriate sample dilutions should be selected such that the final protein concentration lies near the middle of the linear dynamic range of the assay.

It is recommended to prepare 150 µl of sample for each replicate to be assayed. The samples should be diluted with sample diluent and mixed gently.

## Assay protocol

It is recommended that all reagents and materials be equilibrated to 37°C/room temperature prior to the experiment (see Preparation Before The Experiment if you have missed this information).

1. Prepare all reagents and working standards as directed previously.

2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Add 100  $\mu$ l of the standard, samples, or control per well. Add 100  $\mu$ l of the sample diluent buffer into the zero well. At least two replicates of each standard, sample, or control is recommended.
4. Cover with the plate sealer provided and incubate for 120 minutes at RT (or 90 min. at 37 °C).
5. Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
6. Add 100  $\mu$ l of the prepared 1x Biotinylated Anti-Human AHSG antibody to each well.
7. Cover with plate sealer and incubate for 90 minutes at RT (or 60 minutes at 37°C).
8. Wash the plate 3 times with the 1x wash buffer.
  - a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
  - b. Add 300  $\mu$ l of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
  - c. Repeat steps a-b 2 additional times.
9. Add 100  $\mu$ l of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with the plate sealer provided and incubate for 40 minutes at RT (or 30 minutes at 37°C).
10. Wash the plate 5 times with the 1x wash buffer.
  - a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
  - b. Add 300  $\mu$ l of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
  - c. Repeat steps a-b 4 additional times.
11. Add 90  $\mu$ l of Color Developing Reagent to each well. Cover with the plate sealer provided and incubate in the dark for 30 minutes at RT (or 15-25 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.)

12. Add 100  $\mu$ l of Stop Solution to each well. The color should immediately change to yellow.
13. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm.

### Data Analysis

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.

It is recommended that a standard curve be created using computer software to generate a four parameter logistic (4-PL) curve-fit. A free program capable of generating a four parameter logistic (4-PL) curve-fit can be found online at: [www.myassays.com/four-parameter-logistic-curve.assay](http://www.myassays.com/four-parameter-logistic-curve.assay).

Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.

For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

### Background on AHSG

Alpha2-HS glycoprotein (AHSG), also known as fetuin-A, is a plasma protein displaying high-affinity interaction with calcium phosphate, by which ectopic vascular calcification is prevented. The AHSG polymorphism is attributable to the hereditary variation of AHSG and phosphate serum levels, which may affect skeletal development and chronic disorders such as vascular calcification.<sup>1</sup> Human plasma protein alpha 2-HS-glycoprotein (AHSG) is composed of two polypeptide chains, A and B, encoded by a single mRNA. Southern blot analysis of mouse x human somatic cell hybrids has mapped the AHSG gene to human chromosome 3 in the region 3q21----qter. Using a recombinant plasmid containing a 1,538 bp insert spanning the entire AHSG coding region, AHSG was localized to chromosomal bands 3q27----q29 by in situ hybridization.<sup>2</sup>

### 7 Publications Citing This Product

1. PubMed ID: 10.1186/s12865-016-0171-y, Plasma fetuin-A/ $\alpha$ 2-HS-glycoprotein correlates negatively with inflammatory cytokines, chemokines and activation biomarkers in individuals with type-2 diabetes
2. PubMed ID: 10.1016/j.cyto.2016.04.009, Levels of serum pentraxin 3, IL-6, fetuin A and insulin in patients with rheumatoid arthritis
3. PubMed ID: 10.1111/petr.12984, Cardiometabolic risks vary by weight status in pediatric kidney and liver transplant recipients: A cross-sectional, single-center study in the USA