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Human alpha-2-HS- Glycoprotein ELISA Kit

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For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

Thank you for choosing Assaypro.

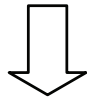
Hinweis/Note:

Der Packungsbeileger dient nur als erste Information.
Der relevante Packungsbeileger liegt der Ware bei.

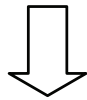
The datasheet is only a first information.
The relevant datasheet is included with the product.

Assay Summary

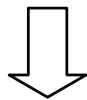
Add 50 μ l of Standard/ Sample per well.
Incubate 2 hours.



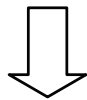
Wash, then add 50 μ l of
Biotinylated Antibody per well.
Incubate 1 hour.



Wash, then add 50 μ l of
SP Conjugate per well.
Incubate 30 minutes.



Wash, then add 50 μ l of
Chromogen Substrate per well.
Incubate 12 minutes.



Add 50 μ l of Stop Solution per well.
Read at 450 nm immediately.

AssayMax Human alpha-2-HS-Glycoprotein (AHSG) ELISA Kit

Catalog No. EG3501-1
Sample Insert/Reference Only

Introduction

The alpha-2-Heremans-Schmid Glycoprotein (AHSG), also known as alpha-2-HS-Glycoprotein, or fetuin-A, is a highly glycosylated plasma protein synthesized in liver and enriched in bone (1). AHSG is an abundant serum protein with powerful calcification inhibitory properties. AHSG deficiency was recently linked to cardiovascular mortality in dialysis patients (2, 3). While increased fetuin-A levels positively correlated with vascular calcification in patients with diabetes and mild to moderate renal impairment, an inverse relationship was observed in dialysis patients. Both chronic inflammation and uremia may contribute to exhausting fetuin-A release in the late stages of kidney disease (4). It has been recently reported AHSG is decreased in the cerebrospinal fluid of patients with Alzheimer's disease (5).

Principle of the Assay

The AssayMax Human alpha-2-HS-Glycoprotein ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human alpha-2-HS-Glycoprotein in plasma, serum, urine, milk, CSF, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures alpha-2-HS-Glycoprotein in less than 4 hours. A polyclonal antibody specific for alpha-2-HS-Glycoprotein has been pre-coated onto a 96-well microplate with removable strips. Alpha-2-HS-Glycoprotein in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for alpha-2-HS-Glycoprotein, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- **Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.**

- **Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.**
- **Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.**
- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acidic solution.

Reagents

- **Human alpha-2-HS-Glycoprotein Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human alpha-2-HS-Glycoprotein.
- **Sealing Tapes:** Each kit contains 3 precut, pressure-sensitive sealing tapes, which can be cut to fit the format of the individual assay.
- **Human alpha-2-HS-Glycoprotein Standard:** Human alpha-2-HS-Glycoprotein in a buffered protein base (800 ng, lyophilized).
- **Biotinylated Human alpha-2-HS-Glycoprotein Antibody (50x):** A 50-fold biotinylated polyclonal antibody against alpha-2-HS-Glycoprotein (140 μ l).
- **MIX Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 μ l).
- **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution:** A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store SP Conjugate and Biotinylated Antibody at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.

- Store Standard at 2-8°C before reconstituting with diluent and at -20°C after reconstituting with diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 μ l, 20-200 μ l, 200-1000 μ l, and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x *g* for 10 minutes. Dilute samples 1:10000 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes and remove serum. Dilute samples 1:10000 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x *g* for 10 minutes. Dilute samples 1:2 with MIX Diluent or within the range of 1x to 20x, and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Milk:** Collect milk using sample tube. Centrifuge samples at 800 x *g* for 10 minutes. Dilute samples 1:2 with MIX Diluent or within the range of 1x to 20x, and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **CSF:** Collect cerebrospinal fluid (CSF) using sample pot. Centrifuge samples at 3000 x *g* for 10 minutes. Dilute samples 1:30 into MIX Diluent and assay. The undiluted samples can be stored at -80°C for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x *g* for 10 minutes to remove debris. Collect supernatants, dilute if necessary and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **MIX Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

Dilute the MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.

- **Human alpha-2-HS-Glycoprotein Standard:** Reconstitute the 800 ng of Human alpha-2-HS-Glycoprotein Standard with 4 ml of MIX Diluent to generate a 200 ng/ml standard solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (200 ng/ml) 1:2 with equal volume of MIX Diluent to produce 100, 50, 25, 12.5, 6.25, and 3.13 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[AHSG] (ng/ml)
P1	Standard (200 ng/ml)	200.0
P2	1 part P1 + 1 part MIX Diluent	100.0
P3	1 part P2 + 1 part MIX Diluent	50.00
P4	1 part P3 + 1 part MIX Diluent	25.00
P5	1 part P4 + 1 part MIX Diluent	12.50
P6	1 part P5 + 1 part MIX Diluent	6.250
P7	1 part P6 + 1 part MIX Diluent	3.125
P8	MIX Diluent	0.000

- **Biotinylated Human alpha-2-HS-Glycoprotein Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with MIX Diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

- Prepare all reagents, working standards, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Human alpha-2-HS-Glycoprotein Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.

- Wash five times with 200 μ l of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 μ l of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 μ l of Biotinylated Human alpha-2-HS-Glycoprotein Antibody to each well and incubate for 1 hour.
- Wash the microplate as described above.
- Add 50 μ l of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 μ l of Chromogen Substrate per well and incubate for about 12 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 μ l of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

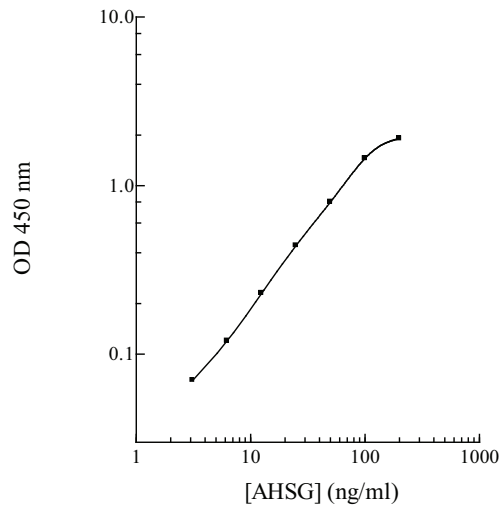
Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.

Human AHSG Standard Curve



Performance Characteristics

- The minimum detectable dose of alpha-2-HS-Glycoprotein is ~ 3 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 5.0% and 7.0% respectively.

Linearity

Sample Dilution	Average Percentage of Expected Value	
	Plasma	Serum
1:5000	92%	94%
1:10000	100%	101%
1:20000	103%	104%

Recovery

Standard Added Value	10 – 100 ng/ml
Recovery %	88 – 110%
Average Recovery %	99%

Cross-Reactivity

Species	% Cross Reactivity
Canine	None
Monkey	None
Mouse	None
Rat	None
Swine	None
Bovine	None
Rabbit	None
Human	100%

References

- (1) Yang F *et al.* (1991) *Bone* 12(1): 7-15
- (2) Merx MW *et al.* (2005) *J Am Soc Nephrol.* Nov; 16(11): 3357-64
- (3) Floege J *et al.* (2004) *Nephrol Dial Transplant.* 5:V59-66
- (4) Ketteler M (2005) *Curr Opin Nephrol Hypertens.* Jul; 14(4): 337-42
- (5) Geroldi D *et al.* (2005) *Neurosci Lett.* 7; 386(3): 176-8

Version 3.5