Datasheet for ABIN612703

# Fetuin A ELISA Kit

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## Overview

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| Quantity: | 96 tests |
| Target: | Fetuin A (AHSG) |
| Reactivity: | Human |
| Method Type: | Sandwich ELISA |
| Minimum Detection Limit: | 3 ng/mL |
| Application: | ELISA |

## Product Details

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| Purpose: | The AssayMax Human Alpha-2-HS-Glycoprotein ELISA kit is designed for detection of human alpha-2-HS-Glycoprotein in plasma, serum, and cell culture supernatants |
| Brand: | AssayMax |
| Sample Type: | Plasma, Cell Culture Supernatant |
| Analytical Method: | Quantitative |
| Detection Method: | Colorimetric |
| Specificity: | Reference Value: The normal blood levels of alpha-2-Heremans-Schmid Glycoprotein (AHSG) range from 300-400 ug/ml. |
| Components: | 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 pre-cut, pressure-sensitive sealing tapes, which can be cut to fit the format of the individual assay. Alpha-2-HS-Glycoprotein Standard: Human alpha-2-HS-Glycoprotein in a buffered protein base (800 ng, lyophilized). Biotinylated alpha-2-HS-Glycoprotein Antibody |

## Product Details

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| | (100x): A 100-fold biotinylated polyclonal antibody against alpha-2-HS-Glycoprotein (80µl). 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 (80µl). 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). |
| Material not included: | Microplate reader capable of measuring absorbance at 450 nm. Pipettes (1-20 µL, 20-200 µL, 200-1000µLand multiple channel). Deionized or distilled reagent grade water. |

## Target Details

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| Target: | Fetuin A (AHSG) |
| Abstract: | AHSG Products |
| Background: | 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. AHSG is an abundant serum protein with powerful calcification inhibitory properties. AHSG deficiency was recently linked to cardiovascular mortality in dialysis patients. 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. It has been recently reported AHSG to be decreased in the cerebrospinal fluid of patients with Alzheimer's disease. |

## Application Details

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| Sample Volume: | 50 µL |
| Assay Time: | < 4 h |
| Plate: | Pre-coated |
| Protocol: | 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 microplate. 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. |

## Application Details

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| Reagent Preparation: | Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. MIx Diluent Concentrate (10x): Dilute the MIx Diluent Concentrate 1:10 with reagent grade water. Store for up to 1 month at 2-8°C. 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 standard solution of 200 ng/ml. 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) twofold with equal volume of MIx Diluent to produce 100, 50, 25, 12.5, 6.25 and 3.13 ng/ml. MIx Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C. Standard Point Dilution [AHSG] (ng/ml) P1 1 part Standard (200 ng/ml) 200.00 P2 1 part P1 + 1 part MIx Diluent 100.00 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 6.25 P7 1 part P6 + 1 part MIx Diluent 3.13 P8 MIx Diluent 0.00 Biotinylated alpha-2-HS-Glycoprotein Antibody (100x): Spin down the biotinylated antibody briefly and dilute the desired amount of the antibody 1:100 with MIx Diluent. Any remaining solution should be frozen at -20°C. Wash Buffer Concentrate (20x): 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. |
| Sample Collection: | Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and assay. Dilute samples 1:10000 into MIx Diluent. Add 5 µl of sample to 495 µl of MIx Diluent (1:100) to make Solution A, then add 5 µl of Solution A to 495 µl of MIx Diluent (1:100) to make a final working solution (1:10000). 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 anticoagulant.) Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and assay. Dilute samples 1:10000 into MIx Diluent. Add 5 µl of sample to 495 µl of MIx Diluent (1:100) to make Solution A, then add 5 µl of Solution A to 495 µl of MIx Diluent (1:100) to make a final working solution (1:10000). The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Cell Culture Supernatants: Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Dilute samples 1:5 into MIx Diluent. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles. 2 |
| 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 - 30 °C). Remove |

## Application Details

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| | excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator. Add 50 µL of standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition. Wash five times with 200 µL of Wash Buffer manually. Invert the plate each time and decant the contents, hit it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µL of Wash Buffer and then invert the plate, decant the contents, hit it 4-5 times on absorbent paper towel to completely remove the liquid. Add 50 µL of Biotinylated alpha-2 HS-Glycoprotein Antibody to each well and incubate for one hour. Wash a microplate as described above. 3 Add 50 µL of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance. Wash a microplate as described above. Add 50 µL of Chromogen Substrate per well and incubate for about 10 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 µL 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. |
| Calculation of Results: | Calculate the mean value of the 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 4-parameter or semi-log curve fit. Determine the unknown sample concentration from the Standard Curve and multiply the sample 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. |
| Assay Precision: | Intra-assay and inter-assay coefficients of variation were 4.9 % and 7.1% respectively. |
| Restrictions: | For Research Use only |

## Handling

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| Handling Advice: | 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 |

## Handling

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| | should determine the optimal dilution factor. Spin down the SP conjugate vial and the biotinylated-antibody vial before opening and using contents. The kit should not be used beyond the expiration date. |
| Storage: | 4 °C/-20 °C |
| Storage Comment: | Store kit at 2-8°C or -20°C upon arrival up to the expiration date. Opened MIx Diluent may be stored for up to 1 month at 2-8°C. Store reconstituted reagents at -20°C or below. Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator. |

## Publications

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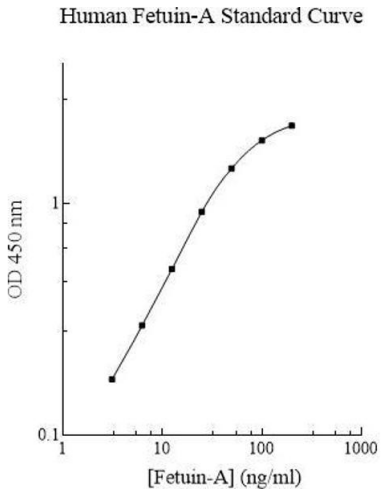
| Product cited in: | Akin, Celik, Altun, Ayca, Diker, Satılmıs, Sahin: "Relationship of fibroblast growth factor 23 and fetuin--a to coronary atherosclerosis." in: **Journal of diabetes and its complications**, Vol. 29, Issue 4, pp. 550-5, (2015) (PubMed).<br><br>Akin, Celik, Ayca, Altun, Diker, Byk, Siriopol, Covic, Kanbay: "Associations of fibroblast growth factor 23 and fetuin-A with coronary plaque burden and plaque composition in young adults." in: **Journal of investigative medicine : the official publication of the American Federation for Clinical Research**, Vol. 63, Issue 4, pp. 613-9, (2015) (PubMed).<br><br>Iliodromiti, Vrachnis, Samoli, Iliodromiti, Pangalos, Drakoulis, Creatsas, Botsis: "Fetuin A concentration in the second trimester amniotic fluid of fetuses with trisomy 21 appears to be lower: phenotypic considerations." in: **Mediators of inflammation**, Vol. 2012, pp. 138971, (2012) (PubMed). |




## ELISA

**Image 1.**