Datasheet for ABIN612706

ORM1 ELISA Kit

1 Image | 1 Publication

Go to Product page

Overview

Quantity:	96 tests
Target:	ORM1
Reactivity:	Human
Method Type:	Sandwich ELISA
Minimum Detection Limit:	5 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Human AGP ELISA (Enzyme-Linked Immunosorbent Assay) kit employs a quantitative sandwich enzyme immunoassay technique that measures cell culture supernatant and urine AGP
Brand:	AssayMax
Sample Type:	Plasma, Cell Culture Supernatant
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	Human AGP Microplate: A 96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human AGP. Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay. Human AGP Standard: Human AGP in a buffered protein base (200 ng, lyophilized). Biotinylated AGP Antibody (30x): A 30-fold biotinylated polyclonal antibody against human AGP (300µl). 1 MIx Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 ml). Wash Buffer

Product Details

	Concentrate (20x): A 20-fold concentrated buffered surfactant (30 ml). Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate (90µl). 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

Target:	ORM1
Alternative Name:	alpha-1-Acid Glycoprotein (ORM1 Products)
Background:	Alpha-1-Acid Glycoprotein (AGP) is an acute-phase protein secreted by the liver which under conditions of inflammation increase several-fold in concentration. An elevated serum level of acute-phase inflammatory markers is associated with an increased risk of cardiovascular disease Urinary orosomucoid excretion rate predicts cardiovascular mortality in patients with Type II diabetes. AGP can be used as a marker for inflammation , chronic alcohol drinking , chronic kidney disease , and asthma.
Pathways:	Response to Growth Hormone Stimulus

Application Details

Sample Volume:	50 µL
Assay Time:	< 4 h
Plate:	Pre-coated
Protocol:	A polyclonal antibody specific for AGP has been pre-coated onto a microplate. AGP in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for AGP, 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.
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 1:10 with reagent grade water. Store for up to 1 month at 2-8°C. Standard Curve: Reconstitute the 200 ng of AGP Standard with 1 ml of MIx Diluent to generate a solution of 200 ng/ml. Allow the standard to sit for 10 minutes with gentle

	agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the standard solution (200 g/ml) 1:2 with 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. Standard Point Dilution [AGP] (ng/ml) P1 Standard (200ng/ml) 200.00 P2 1 part P1 + 1 parts MIx Diluent 100.00 P3 1 part P2 + 1 parts MIx Diluent 50.00 P4 1 part P3 + 1 parts MIx Diluent 25.00 P5 1 part P4 + 1 parts MIx Diluent 12.50 P6 1 part P5 + 1 parts MIx Diluent 6.25 P7 1 part P6 + 1 parts MIx Diluent 3.13 P8 MIx Diluent 0.00 Biotinylated AGP Antibody (30x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:30 with MIx Diluent. Any remaining solution should be frozen at -20°C. Wash Buffer Concentrate (10x): Dilute the Wash Buffer Concentrate 1:10 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:	Cell Culture Supernatants: Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store the remaining samples at -20°C or below. Avoid repeated freeze-thaw cycles. Urine: Collect urine using sample pot. Centrifuge samples at 600 x g for 10 minutes and assay. Urine dilution is suggested at 1:100 in MIx Diluent, however, the user should determine the optimal dilution factor. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
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 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. Invert the plate and decant the contents, and hit it 4-5 times on absorbent paper towel to complete remove liquid at each step. Add 50 µL of Biotinylated AGP Antibody to each well and incubate for one hour. Wash five times with 200 µL of Wash Buffer as above. 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 five times with 200 µL of Wash Buffer as 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 the 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. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10

Application Details

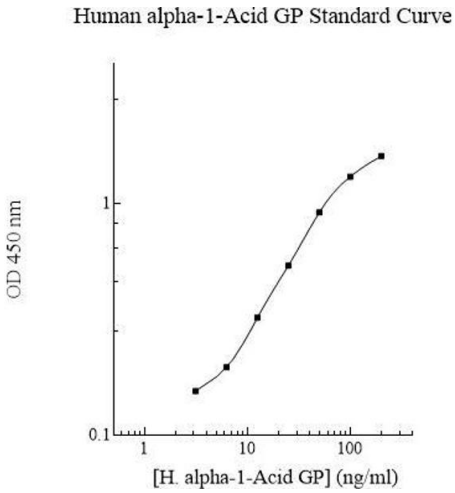
	minutes, which will reduce the readings.
Calculation of Results:	Calculate the mean value of the duplicate or triplicate readings for each standard and sample. To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on 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.
Assay Precision:	Intra-assay and inter-assay coefficients of variation were 4.3% and 7.0% respectively.
Restrictions:	For Research Use only

Handling

Handling Advice:	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

Product cited in:	Xu, Lian, Zhao, Zhao, Chen, Zhang, Guo, Zhang, Zhou, Xue, Pang, Zhao, Tong: "Structural modulation of gut microbiota during alleviation of type 2 diabetes with a Chinese herbal formula." in: **The ISME journal**, Vol. 9, Issue 3, pp. 552-62, (2015) (PubMed).



ELISA

Image 1.

