ELISA kits available from ADI:

Human: Adiponectin (Acrp30 and gAcrp30), Albumin, Aldosterone, AFP, beta-amyloid 1-40/42, Angiogenin, Angiopoietin-2, beta-2M, BMP-7, C-peptide, CRP, Cox-2, Ferritin, PSA, fPSA, GH, IgG, IgM, IgE, IgG1, IgG4, Insulin, NSE, CA125, CA199, CA242, PAP, Resistin, SHBG, LH, FSH, TSH, T3, T4, and Steroid ELISA kits (cortisol, estradiol, testosterone, progesterone).

Monkey: IgM, IgG, IgA, IgE

Rat: Albumin, CRP, IgG, IgM, Alpha-1- Acid glycoprotein

Mouse: Albumin, IgA, IgG, IgG1, IgG2a, IgG2b, IgG3, IgE, IgM, Leptin, Resistin, Acrp30, CRP, Haptoglobin, TNF-alpha
Autoimmune Antibody detection kits for ANA, ssDNA, dsDNA, Histone, Sm, RNP, SSA, SSB, Scl70, Ovalbumin, Cardiolipin, CIC

Chicken: IgG, IgM, IgY, Ovalbumin

T**urkey**: IgG

Bovine: Albumin, IgG, IgM, Lactoferrin, Transferrin

Pig: Albumin, IgG, IgM

Dog: CRP, IgG, IgM

Cat: IgG, IgM

Goat: IgG

Rabbit: CRP, IgG

Sheep: IgG

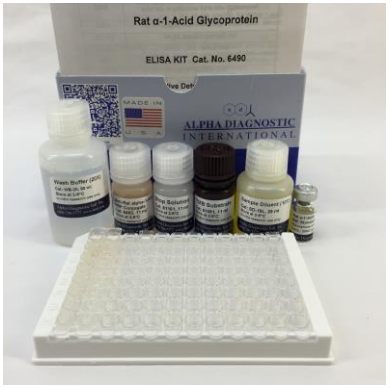
See Details at the web site or Contact ADI

Instruction Manual No. M-6490

Rat α -1-Acid Glycoprotein (AGP)

ELISA KIT # 6490, 96 tests

For Quantitative Determination of α-1-Acid Glycoprotein (AGP) in Rat Serum, plasma or other biological fluids



For In Vitro Research Use Only (RUO)

ALPHA DIAGNOSTIC INTERNATIONAL

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Rat α -1-AGP ELISA KIT Cat. No. 6490, 96 tests

Kit Components, 96 tests	#
Anti-Rat α-1-AGP coated strip plate (8 wells x 12 strips), #6491	1 plate
Rat α-1-AGP Reference **Standard, lyophilized** *Reconstitute with dH_20 according to vial label,* #6492	6492
Anti-Rat -α-1-AGP-**HRP Conjugate**, 11 ml, # 6 4 9 3	1 bottle
10x **Sample Diluent**, 25 ml, #SD-10L	1 bottle
Wash Buffer (20x), 50 ml, # WB-20	1 bottle
TMB Substrate, 11 ml, # 81091	1 bottle
Stop solution, 11 ml, # 81101	1 bottle
Instruction Manual, #M-6490	1

Intended Use:

Rat α-1-Acid Glycoprotein ELISA kit is a sandwich assay for the measurement of l Rat α-1-Acid Glycoprotein in rat serum, plasma or in other biological fluids. For in vitro research use only (RUO).

INTRODUCTION

Orosomucoid (ORM) or alpha-1-acid glycoprotein (α1AGp, AGP or AAG) is an acute phase (acute phase protein) plasma alpha-globulin glycoprotein and is modulated by two polymorphic genes. It is synthesized primarily in hepatocytes and has a normal plasma concentration between 0.6-1.2 mg/mL (1-3% plasma protein). Plasma levels are affected by pregnancy, burns, certain drugs, and certain diseases, particularly HIV. Alpha-1-acid glycoprotein has been identified as one of four potentially useful circulating biomarkers for estimating the five-year risk of all-cause mortality (the other three are albumin, very low-density lipoprotein particle size, and citrate).

Alpha-1-Acid Glycoprotein (AGP) is synthesized by the liver and subsequently secreted into the plasma. Synthesis is controlled by glucocorticoids, interleukin-1 and interleukin-6. The protein appears to function in modulating the activity between blood cells and endothelial cells. Together with haptoglobin and C-reactive protein, AGP also

PERFORMANCE CHARACTERISTICS

Detection Limit: The minimum AGP concentration detectable using this assay is below 1 ng/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

Expected Values: Rat AGP levels in serum may vary from 1 ug/ml to above 1 mg/ml during acute phase responses. Each laboratory should establish testing ranges for the animal population being investigated.

Specificity: The antibodies used in this kit are specific for alpha-1-acid glycoprotein and have shown no cross-reactivity with other serum proteins.

Species Crossreactivity: Cross-reactivity was tested with animal sera at dilutions of 1:10. Significant reactivity was observed with mouse serum, and low reactivity with monkey, goat and sheep. Rabbit, human, bovine, hamster and cat sera have insignificant reactivity in rat AGP ELISA.

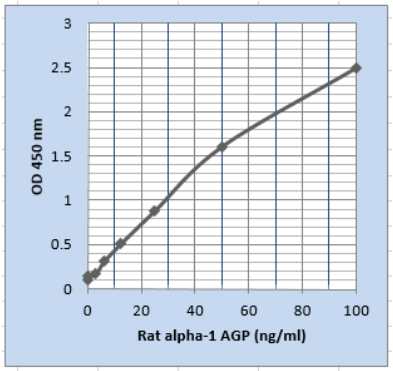
References

Colombo S (2006) *Clin. Pharmacol. Ther.* **80** (4): 307–18; Dietrcih JW (2012) Thyroid Res. 2012;2012:351864; Ricca GA (1981) JBC 256, 11199-11202; Liao YCJ (1985) Mol. Cell Biol. 5, 3634-3639; Reinke R (1985) JBC 260, 4397-4403

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A_{450} nm
A1, A2	Sample Diluent (blank)	0.139
B1, B2	**Standard A** 3.13 ng/ml	0.167
C1, C2	**Standard B** 6.25 ng/ml	0.307
D1, D2	**Standard C** 12.5 ng/ml	0.509
E1, E2	**Standard D** 25 ng/ml	0.876
F1, F2	**Standard E** 50 ng/ml	1.601
G1, G2	**Standard F** 100 ng/ml	2.488
H1, H2	**Sample 1** 1:50,000 dilution	1.51

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



6-ADI_Alpha-Graph

A typical assay Standard Curve (do not use this for calculating sample values)

being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 2-8^{o}C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each wells the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Draw the standard curve on semi-log graph paper by plotting net absorbance values of standards against appropriate AGP concentrations. Read off the AGP concentrations of the control and patient samples. Multiply the values by the dilution factor of the samples. If samples were diluted 1:50k then the values must be multiplied by 50,000 and results are expressed as mg/ml.

regulates the extra-vasculation of the cells during infection and inflammation. AGP is a major acute phase reactant, both in human and in rat. Its concentration in blood plasma is elevated 6-60 fold during acute inflammation, such as trauma, malignancies, myocardial infarction, rheumatoid arthritis, bacterial infections, after major surgery, etc, and can be used for the diagnosis of inflammatory conditions.

Alpha Diagnostic Intl's AGP ELISA kit is a highly sensitive sandwich type assay for the measurement of AGP in serum. The assay can be adapted to measure rat α-1-Acid in other biological fluids such as plasma and urine, and in culture medium.

Human AGP/ Alpha-1-acid glycoprotein/ORM1: protein accession #P02763 (201-aa)
Rat AGP: P02764 (205-aa)
Mouse AGP: P21350 (207-aa)

Mouse, rat, and human are 100%, 70%, and 47.8% conserved, respectively.

PRINCIPLE OF THE TEST

Rat AGP ELISA kit is based on binding of Rat AGP from samples to two antibodies, one immobilized on the microtiter well plates, and other conjugated to the enzyme horseradish peroxidase. After a washing step, chromogenic substrate is added and colors developed. The enzymatic reaction (color) is directly proportional to the amount of AGP present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm. and the concentration of AGP in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-1000 ul) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plates Reader.

PRECAUTIONS AND SAFETY INSTRUCTIONS

The Rat AGP ELISA Kit is for research use only.
Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

MSDS for TMB, sulfuric acid, if not already on file, can be requested or obtained from the ADI website.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow clotting, and separating the serum by centrifugation at room temperature. Do not heat inactivate the serum. If sera can not be immediately assayed , store frozen for up to six months. Avoid repeated freezing and thawing of samples. It is also possible to use plasma for testing.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{o}C until the expiration date printed on the label. After opening the kit components, the shelf life is approximately 2 months.

REAGENT PREPARATION

1. Dilute the Sample Diluent 1:10 with water (10 ml diluent in 90ml water). Dilute only the required reagent. Store diluted solution at 2-8^{o} C for 3-4 days.

2. The Wash Buffer is a 20x stock. Dilute the entire 50 ml with distilled or deionized water to 1 L total volume. Store at room temperature for the entire use of the kit.

AGP Standard Preparation

Reconstitute the lyophilized Reference Standard with the amount of distilled water indicated on the vial label (usually ~1 ml). The stock concentration will be 2 ug/ml (2000 ng/ml). Prepare 10-100 ul vials of the stock and store frozen at -20oC or below.

Working standards are prepared by performing 2-fold serial dilutions. **Store unused Reference Standard at -20°C.**

Prepare other liquid standards using the following dilution scheme.

Std	Diluent	**AGP Stds (**Concn)
10 ul of stock std (2000 ng/ml)	1990 uL	**F (**100 ng/ml)
250 uL of std F.	250 uL	**E (**50 ng/ml)
250 uL of std F.	250 uL	**D (**25 ng/ml)
250 uL of std F.	250 uL	**C (**12.5 ng/ml)
250 uL of std F.	250 uL	**B (**6.25 ng/ml)
250 uL of std F.	250 uL	**A (**3.13 ng/ml)

Do not store diluted standards beyond the assay dates. Always use freshly diluted standards.

DILUTION OF SAMPLES

Samples containing more than 100 ng/ml AGP should be further diluted and re-tested. The results obtained should be multiplied by the appropriate dilution factor. It is possible to use normal saline or PBS for sample dilution if larger volumes of samples are taken for dilution or if more sample diluent is required.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

Diluting the rat serum samples ~1:50,000 (use 1x Sample Diluent) will bring most samples into the testing range. For those testing out of the range dilute accordingly.

1. Label or mark the microtiter well strips to be used on the plate.

2. Pipet **100 ul** standards and diluted samples into appropriate wells.

3. Mix gently, and incubate on an orbital micro-plate shaker at 100-150 rpm room temperature (18-25 oC) for **45 minutes.**

4. **NOTES:** Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is Wash the wells **5 times** with 350 ul of **1x wash buffer**.

5. Pipette **100 ul of Ab-enzyme conjugate** into each well. Mix gently, and incubate on an orbital micro-plate shaker at 100-150 rpm room temperature (18-25 oC) for **45 minutes** at room temperature.

6. Add **100 ul of TMB Substrate** into each well. Mix gently. Cover the plate and incubate on an orbital micro-plate shaker at 100-150 rpm room temperature (18-25 oC) for **20 minutes** at room temperature. Blue color develops.

7. Stop the reaction by adding **100 ul of stop solution** to all wells. Mix gently. Blue color turns yellow.

8. Measure the absorbance at **450 nm** using an ELISA reader. Color is stable for at least 30 minutes after stopping.

Notes

Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is