Instruction Manual No. M-6490

Rat α-1-Acid Glycoprotein

ELISA KIT Cat. No. 6490

For Quantitative Determination of α-1-Acid Glycoprotein in Rat Serum

Rat α-1-AGP ELISA KIT Cat. No. 6490

Kit Components, 96 tests	Cat #
Anti-Rat α-1-AGP coated strip plate (8 wells x 12 strips)	6491
Rat α-1-AGP Reference Standard, lyophilized *Reconstitute with dH_20 according to vial label*	6492
Anti-Rat α-1-AGP-HRP Conjugate, 11 ml	6493
10x Sample Diluent, 25 ml	SD-10L
Wash Buffer (20x), 50 ml	WB-20
TMB Substrate, 11 ml	81091
Stop solution, 11 ml	81101
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NTRODUCTION

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 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.

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.

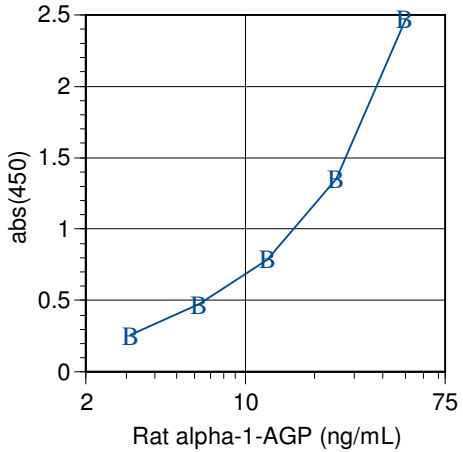
Specificity: The antibodies used in this kit are specific for alpha-1-acid glycoprotein and have shown no cross-reactivity with other serum proteins.

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. Reactivities of rabbit, human, bovine, hamster and cat sera were insignificant.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean $A_{450\ nm}$	Calculated Concn
A1, A2	**Negative Diluent Control**	0.106	
B1, B2	**Standard A** 3.13 ng/ml	0.258	
C1, C2	**Standard B** 6.25 ng/ml	0.474	
D1, D2	**Standard C** 12.5 ng/ml	0.786	
E1, E2	**Standard D** 25 ng/ml	1.351	
F1, F2	**Standard E** 50 ng/ml	2.467	
G1, G2	**Sample 1** 1:500 dilution	1.51	27 ng/ml = **13.5** ug/ml

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.



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

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.

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.

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.

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

1. Reconstitute the lyophilized Reference Standard with the amount of distilled water indicated on the vial label. The stock concentration will be 2 ug/ml. Store unused Reference Standard at -20°C.
2. Prepare liquid standards using the following dilution scheme:

Rat AGP			Diluent		Final Concn		Final Volume
Concn		Volume					
	2000 ng/ml	15 uL	+	585 uL	**E**	50 ng/ml	350 uL
E	50 ng/ml	250 uL	+	250 uL	**D**	25 ng/ml	250 uL
D	25 ng/ml	250 uL	+	250 uL	**C**	12.5 ng/ml	250 uL
C	12.5 ng/ml	250 uL	+	250 uL	**B**	6.25 ng/ml	250 uL
B	6.25 ng/ml	250 uL	+	250 uL	**A**	3.13 ng/ml	500 uL

Diluting the rat serum samples 1:1000-1:50,000 (use 1x Sample Diluent) will bring most samples into the testing range. For those testing out of the range dilute accordingly.

3. Label or mark the microtiter well strips to be used on the plate.
4. Dispense 200-300 uL of wash buffer to all wells. Let stand for 5-15 minutes, then discard or aspirate the solution. The step should be done just before adding the samples; do not allow the wells to dry at any time during the assay.
5. Pipet 100 ul standards and diluted samples into appropriate wells.
6. Mix gently, and incubate at room temperature for 60 minutes.
7. Wash the wells 3 times with 300 ul of 1x wash buffer.
8. Pipette 100 ul of Ab-enzyme conjugate into each well. Mix gently, and incubate for 45 minutes at room temperature.

9. Add 100 ul of TMB Substrate into each well. Mix gently. Cover the plate and incubate for 20 minutes at room temperature. Blue color develops.
10. Stop the reaction by adding 100 ul of stop solution to all wells. Mix gently. Blue color turns yellow.
11. Measure the absorbance at 450 nm using an ELISA reader. Color is stable for at least 30 minutes after stopping.

NOTES: Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. 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 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.

DILUTION OF SAMPLES

Samples containing more than 50 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.

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.