

RAT α -1-ACID GLYCOPROTEIN (α -1-AGP) ELISA TEST KIT

Life Diagnostics, Inc., Catalog Number: 2510-2

Enzyme Immunoassay for the Quantitative Determination of Rat α -1-Acid Glycoprotein Protein (α -1-AGP) in Serum

INTRODUCTION

α -1-AGP is an acute phase protein that is elevated in serum as a result of injury, infection or disease. Studies performed at Life Diagnostics Inc., have demonstrated a ten fold increase in α -1-AGP in rats with chronic disease.

PRINCIPLE OF THE TEST

The rat α -1-AGP ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses affinity purified anti-rat α -1-AGP antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-rat α -1-AGP antibodies for detection. The test sample is diluted and incubated in the microtiter wells for 45 minutes. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 45 minutes. This results in α -1-AGP molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies and TMB Reagent is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. The concentration of α -1-AGP is proportional to the optical density of the test sample.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-rat α -1-AGP antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8)
- Enzyme Conjugate Reagent, 11 ml
- Reference standard (lyophilized), containing 2 μ g/ml rat α -1-AGP when reconstituted as detailed on the vial label
- 10x Diluent (25 ml)
- 20x Wash Solution (50 ml)
- TMB Reagent (One-Step) 11 ml
- Stop Solution (1N HCl), 11 ml

Materials required but not provided:

- Precision pipettes and tips.
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer.
- Absorbent paper or paper towels
- Micro-Plate incubator/shaker with an approximate mixing speed of 150 rpm
- A microtiter plate reader at 450 nm wavelength, with a bandwidth of 10 nm or less and an OD range of 0-4 OD
- Graph paper (PC graphing software is optional)

STORAGE OF TEST KIT AND INSTRUMENTATION

The unused kit should be stored at 2-8°C and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable for six months from the date of purchase.

GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature (18- 25°C) before use.
2. Serum or plasma samples should be diluted ~50,000 fold with 1x diluent in order to obtain values within the standard range.

DILUENT PREPARATION

The diluent is provided as a 10x stock. Prior to use estimate the final volume of diluent required for your assay and dilute one (1) volume of the 10x stock with nine (9) volumes of distilled or deionized water.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. The rat α -1-AGP standard is provided as a lyophilized stock. Add the volume of distilled or de-ionized water indicated on the vial label and mix gently until dissolved to obtain a 2 μ g/ml rat α -1-AGP stock (*the reconstituted standard remains stable for at least 10 days at 2-8°C but should be aliquoted and frozen at -20°C after reconstitution if use beyond this time is intended*).
2. Label 8 polypropylene or glass tubes as 100, 50, 25, 12.5, 6.25, 3.125, 1.56 and 0 ng/ml
3. Dispense 950 μ l of diluent into the tube labeled 100 ng/ml and 300 μ l of diluent into the remaining tubes.
4. Pipette 50 μ l of the 2 μ g/ml α -1-AGP standard into the tube labeled 100 ng/ml and mix. This provides the working 100 ng/ml α -1-AGP standard.
5. Prepare a 50 ng/ml standard by diluting and mixing 300 μ l of the 100 ng/ml standard with 300 μ l of diluent in the tube labeled 50 ng/ml. Similarly prepare the 25, 12.5, 6.25, 3.125, and 1.56 ng/ml standards by serial dilution.

SAMPLE PREPARATION

General Note: Our studies find that α -1-AGP may be present in rat serum at concentrations of 0.05 to 1 mg/ml or greater. In order to obtain values within the range of the standard curve we suggest that samples initially be diluted 50,000 fold using the following procedure for each sample to be tested:

1. Dispense 998 μ l and 495 μ l of 1x diluent into separate tubes.
2. Pipette and mix 2 μ l of the serum/plasma sample into the tube containing 998 μ l of diluent. This provides a 500 fold diluted sample.

- Mix 5 μl of the 500 fold diluted sample with the 495 μl of diluent in the second tube. This provides a 50,000 fold dilution of the sample.
- Repeat this procedure for each sample to be tested.

ASSAY PROCEDURE

- Secure the desired number of coated wells in the holder.
- Dispense 100 μl of standards and samples into the wells (we recommend that samples be tested in duplicate).
- Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes.
- Remove the incubation mixture by flicking plate contents into an appropriate Bio-waste container.
- Wash and empty the microtiter wells 5 times with 1x wash solution. This may be performed using either a plate washer (350 μl /well) or with a squirt bottle. The entire wash procedure should be performed as quickly as possible.
- Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
- Add 100 μl of enzyme conjugate reagent into each well.
- Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes.
- Wash as detailed in 4 and 5 above.
- Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
- Dispense 100 μl of TMB Reagent into each well.
- Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 20 minutes.
- Stop the reaction by adding 100 μl of Stop Solution to each well.
- Gently mix. It is important to make sure that all the blue color changes to yellow.
- Read the optical density at 450 nm with a microtiter plate reader within 15 minutes.

CALCULATION OF RESULTS

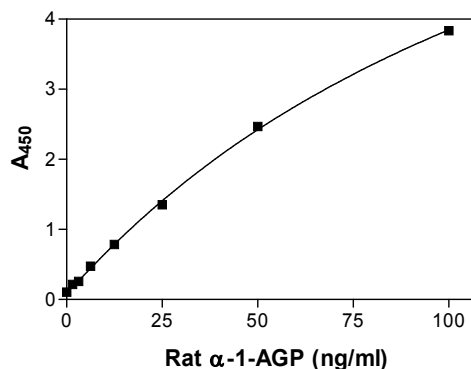
- Calculate the average absorbance values (A_{450}) for each set of reference standards, and samples.
- Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
- Using the mean absorbance value for each sample, determine the corresponding concentration of α -1-AGP in ng/ml from the standard curve.
- Multiply the derived concentrations by the dilution factor to determine the actual concentration of α -1-AGP in the serum/plasma sample.
- If available, PC graphing software may be used for the above steps.
- If the OD_{450} values of samples fall outside the standard curve when tested at a dilution of 50,000. Samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve with optical density readings at 450nm on the Y axis against α -1-AGP concentrations on the X axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and standard curve in each experiment.

α -1-AGP (ng/ml)	Absorbance (450 nm)
100	3.836
50	2.467
25	1.351
12.5	0.786
6.25	0.474
3.13	0.258
1.56	0.214
0	0.106

Typical Rat α -1-AGP Standard Curve



LIMITATIONS OF THE PROCEDURE

- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
- The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.