



Orm1 (Rat) ELISA Kit

Catalog Number KA2133

96 assay

Version: 03

Intended for research use only

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Introduction

Intended Use

The Orm1 (Rat) ELISA Kits are a highly sensitive two-site enzyme linked immunoassay (ELISA) for measuring Alpha 1-Acid Glycoprotein in biological samples of rats.

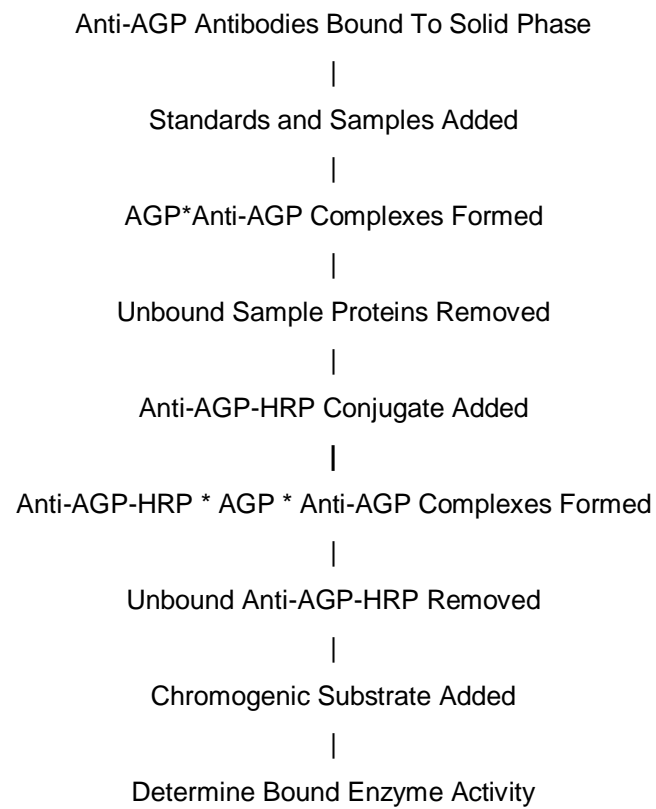
Background

Acute phase proteins are plasma proteins which increase in concentration following infection, inflammation or trauma. The first acute phase protein to be recognized was discovered in humans by Tillet and Frances in 1930¹. Alpha-1-glycoprotein (AGP) is a major acute phase reactant, both in man and in rat. Its concentration in blood plasma is elevated during acute inflammation, such as rheumatoid arthritis, bacterial infections, etc, and can be used for the diagnosis of inflammatory conditions.

Principle of the Assay

The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the Alpha 1-Acid Glycoprotein present in samples reacts with the anti-Alpha 1-Acid Glycoprotein antibodies which have been adsorbed to the surface of polystyrene microtitre wells. After the removal of unbound proteins by washing, anti-AGP antibodies conjugated with horseradish peroxidase (HRP), are added. These enzyme-labeled antibodies form complexes with the previously bound AGP. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme varies directly with the concentration of AGP in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of AGP in the test sample. The quantity of AGP in the test sample can be interpolated from the standard curve constructed from the standards, and corrected for sample dilution.

Figure 1.



General Information

Materials Supplied

List of component

Component	Amount
Diluent Concentrate (Running Buffer): One bottle containing a 5X concentrated diluent running buffer	50 mL
Wash Solution Concentrate: One bottle containing a 20X concentrated wash solution.	50 mL
Enzyme-Antibody Conjugate 100X: One vial containing affinity-purified anti-Rat Alpha 1-Acid Glycoprotein antibody conjugated with horseradish peroxidase in a stabilizing buffer	150 µL
Chromogen-Substrate Solution: One vial containing 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide in citric acid buffer at pH 3.3.	12 mL
Stop Solution: One vial containing 0.3 M sulfuric acid. WARNING: Avoid contact with skin.	12 mL
Anti- Rat Alpha 1-Acid Glycoprotein ELISA Micro Plate: Twelve removable eight (8) well micro well strips in well holder frame. Each well is coated with affinity-purified anti-Rat AGP.	96 wells
Rat Alpha 1-Acid Glycoprotein Calibrator: One vial containing a lyophilized Rat Alpha 1-Acid Glycoprotein calibrator.	1 vial

Storage Instruction

The expiration date for the package is stated on the box label.

1. Diluent

The 5X Diluent Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions should be stored at 4-8°C.

2. Wash Solution

The 20X Wash Solution Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions can be stored at room temperature (16-25°C) or at 4-8°C.

3. Enzyme-Antibody Conjugate

Undiluted horseradish peroxidase anti-AGP conjugate should be stored at 4-8°C and diluted immediately prior to use. The working conjugate solution is stable for up to 1 hour when stored in the dark.

4. Chromogen-Substrate Solution

The Substrate Solution should be stored at 4-8°C and is stable until the expiration date.

5. Stop Solution

The Stop Solution should be stored at 4-8°C and is stable until the expiration date.

6. Anti- Rat Alpha 1-Acid Glycoprotein ELISA Micro Plate

Anti-Rat AGP coated wells are stable until the expiration date, and should be stored at 4-8°C in the sealed foil pouch with desiccant pack.

7. Rat Alpha 1-Acid Glycoprotein Calibrator

The lyophilized Rat Alpha 1-Acid Glycoprotein calibrator should be stored at 4°C or frozen until reconstituted. The reconstituted calibrator should be aliquoted and stored frozen (Avoid multiple freeze-thaw cycles). The working standard solutions should be prepared immediately prior to use and are stable for up to 8 hours.

Materials Required but Not Supplied

- ✓ Precision pipettes (2 µL to 200 µL) for making and dispensing dilutions
- ✓ Test tubes
- ✓ Microplate washer/aspirator
- ✓ Distilled or Deionized H₂O
- ✓ Microtitre plate reader
- ✓ Assorted glassware for the preparation of reagents and buffer solutions
- ✓ Timer

Precautions for Use

✓ Precautions

For any sample that might contain pathogens, care must be taken to prevent contact with open wounds.

✓ Additives and Preservatives

No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.

✓ Known interfering substances

Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.

✓ Limitation of the procedure

- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice.
- Factors that might affect the performance of the assay include proper instrument function, cleanliness of glassware, quality of distilled or deionized water, and accuracy of reagent and sample pipettings, washing technique, incubation time or temperature.
- Do not mix or substitute reagents with those from other lots or sources.

Assay Protocol

Reagent Preparation

✓ **Diluent Concentrate**

The Diluent solution supplied is a 5X Concentrate and must be diluted 1/5 with distilled or deionized water (1 part buffer concentrate, 4 parts dH₂O).

✓ **Wash Solution Concentrate**

The Wash Solution supplied is a 20X concentrate and must be diluted 1/20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH₂O). Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals.

✓ **Enzyme-Antibody Conjugate**

Calculate the required amount of working conjugate solution for each microtiter plate test strip by adding 10 µL Enzyme-Antibody Conjugate to 990 µL of 1X Diluent for each test strip to be used for testing. Mix uniformly, but gently. Avoid foaming.

✓ **Chromogen-Substrate Solution**

Ready to use as supplied.

✓ **Stop Solution**

Ready to use as supplied.

✓ **Anti-Rat Alpha 1-Acid Glycoprotein ELISA Micro Plate**

Ready to use as supplied. Unseal Microtiter Pouch and remove plate from pouch. Remove all strips and wells that will not be used in the assay and place back in pouch and re-seal along with desiccant.

✓ **Rat Alpha 1-Acid Glycoprotein Calibrator**

Add 1.0 ml of distilled or de-ionized water to the Rat Alpha 1-Acid Glycoprotein calibrator and mix gently until dissolved. The calibrator is now at a concentration of 2.04 µg/ml (the reconstituted calibrator should be aliquoted and frozen if future use is intended). Rat Alpha 1-Acid Glycoprotein standards need to be prepared immediately prior to use (See chart below). Mix well between each step. Avoid foaming.

Standard	ng/mL	Volume added to 1X Diluent	Volume of 1X Diluent
6	400	100 µL Rat AGP Calibrator	410 µL
5	200	250 µL standard 6	250 µL
4	100	250 µL standard 5	250 µL
3	50	250 µL standard 4	250 µL
2	25	250 µL standard 3	250 µL
1	12.5	250 µL standard 2	250 µL
0	0		500 µL

Sample Preparation

✓ Specimen Collection and Handling

Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. For plasma samples, blood should be collected into a container with an anticoagulant and then centrifuged. Care should be taken to minimize hemolysis, excessive hemolysis can impact your results. Assay immediately or aliquot and store samples at -20°C. Avoid repeated freezing-thaw cycles.

✓ Dilution of Samples

The assay for quantification of Alpha 1-Acid Glycoprotein in samples requires that each test sample be diluted before use. For a single step determination a dilution of 1/3,000 is appropriate for most serum/plasma samples. For absolute quantification, samples that yield results outside the range of the standard curve, a lesser or greater dilution might be required. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

- To prepare a 1/3,000 dilution of sample, transfer 5 µL of sample to 495 µL of 1X diluent. This gives you a 1/100 dilution. Next, dilute the 1/100 samples by transferring 10 µL, to 290 µL of 1X diluent. You now have a 1/3,000 dilution of your sample. Mix thoroughly at each stage.

Assay Procedure

1. Bring all reagents to room temperature before use.
2. Pipette 100 µL of
 - Standard 0 (0.0 ng/mL) in duplicate
 - Standard 1 (12.5 ng/mL) in duplicate
 - Standard 2 (25 ng/mL) in duplicate
 - Standard 3 (50 ng/mL) in duplicate
 - Standard 4 (100 ng/mL) in duplicate
 - Standard 5 (200 ng/mL) in duplicate
 - Standard 6 (400 ng/mL) in duplicate
3. Pipette 100 µL of sample (in duplicate) into predesignated wells.
4. Incubate the microtiter plate at room temperature for sixty (60 ± 2) minutes. Keep plate covered and level during incubation.
5. Following incubation, aspirate the contents of the wells.
6. Completely fill each well with appropriately diluted Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with wash buffer, invert the plate then pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.

7. Pipette 100 μ L of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at room temperature for ten (10 ± 2) minutes. Keep plate covered in the dark and level during incubation.
8. Wash and blot the wells as described in Steps 5/6.
9. Pipette 100 μ L of TMB Substrate Solution into each well.
10. Incubate in the dark at room temperature for precisely ten (10) minutes.
11. After ten minutes, add 100 μ L of Stop Solution to each well.
12. Determine the absorbance at 450 nm of the contents of each well. Calibrate the plate reader to manufacture's specifications.

✓ **Stability of the final reaction mixture**

The absorbance of the final reaction mixture can be measured up to 2 hours after the addition of the Stop Solution. However, good laboratory practice dictates that the measurement be made as soon as possible.

Data Analysis

Calculation of Results

1. Subtract the average background value from the test values for each sample.
2. Using the results observed for the standards construct a Standard curve. The appropriate curve fit is that of a four-parameter logistics curve. A second order polynomial (quadratic) or other curve fits may also be used.
3. Interpolate test sample values from the standard curve. Correct for sera dilution factor to arrive at the Alpha 1-Acid Glycoprotein concentration in original sample.

Performance characteristics

✓ Indications of instability

If the test is performing correctly, the results observed with the standard solutions should be within 20% of the expected values.

Resources

Plate Layout

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