ORM1 (Chicken) ELISA Kit

Catalog Number KA2069

96 assays

Version: 02

Intended for research use only

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Introduction

Intended Use

The ORM1 (Chicken) ELISA Kit is a highly sensitive two-site enzyme-linked immunoassay (ELISA) for measuring AGP in biological fluids of chickens.

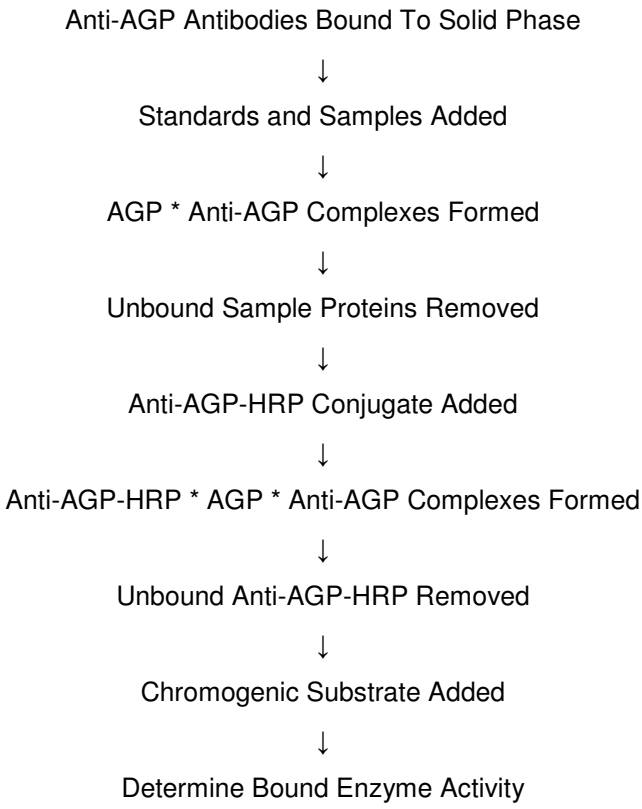
Background

AGP is a 52 kDA serine protease inhibitor (serpin) in blood, which protects tissue from enzymes from inflammatory cells, especially elastase. In certain acute phase inflammatory reactions, AGP is elevated in order to limit the damage caused by activated neutrophil granulocytes and their enzyme elastase. Disorders of AGP include AGP deficiency, a hereditary disorder that can lead to severe tissue breakdown during inflammation. This may result in pulmonary emphysema and liver cirrhosis, in severe cases. Genetic variants of AGP do occur.

Principle of the Assay

The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the AGP present in the sample reacts with the anti-AGP antibody which has been adsorbed to the surface of polystyrene microtiter wells. After the removal of unbound sample proteins by washing, anti-AGP antibody conjugated with horseradish peroxidase (HRP) is added. This HRP-conjugated antibody forms a complex 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 is proportional to 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 calibration curve constructed from the calibrators, and corrected for sample dilution.

Figure 1.


Anti-AGP Antibodies Bound To Solid Phase
↓
Standards and Samples Added
↓
AGP * Anti-AGP Complexes Formed
↓
Unbound Sample Proteins Removed
↓
Anti-AGP-HRP Conjugate Added
↓
Anti-AGP-HRP * AGP * Anti-AGP Complexes Formed
↓
Unbound Anti-AGP-HRP Removed
↓
Chromogenic Substrate Added
↓
Determine Bound Enzyme Activity

General Information

Materials Supplied

List of component

Component	Amount
Diluent Concentrate (Running Buffer): One bottle containing 5X concentrated diluent running buffer.	50 mL
Wash Solution Concentrate: One bottle containing 20X concentrated wash solution.	50 mL
Enzyme-Antibody Conjugate: One vial containing 100X concentrated affinity-purified anti-chicken AGP antibody conjugated with HRP in stabilizing buffer.	150 µL
Chromogenic Substrate Solution: One vial containing 3,3',5,5'-tetramethybenzidine (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- chicken AGP ELISA micro plate: Twelve removable eight (8) well strips in well holder frame. Wells are coated with affinity-purified anti- chicken AGP.	96 wells
Chicken AGP Calibrator: One vial containing Chicken AGP Calibrator.	1 vial

Storage Instruction

- Complete Kit
  The expiration date for the kit is stated on the outer label.
- 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℃.
- 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℃) or at 4-8℃.
- Enzyme-Antibody Conjugate
  Undiluted anti-AGP-HRP conjugate should be stored at 4-8℃ and diluted immediately prior to use. The working conjugate solution is stable for up to 1 hour when stored in the dark.
- Chromogen-Substrate Solution
  The TMB Substrate Solution should be stored at 4-8℃ and is stable until the expiration date.
- Stop Solution
  The Stop Solution should be stored at 4-8℃ and is stable until the expiration date.
- Microtiter Plate
  Anti-chicken AGP coated wells are stable until the expiration date and should be stored at 4-8℃ in the sealed foil pouch with a desiccant pack.

- Chicken AGP Calibrator

  Long Term Storage: Upon receipt, aliquot the calibrator and store them frozen. They will be stable until expiration date. Short Term Storage: the calibrator is stable for up to 14 days at 4ºC. 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 H_2O
- ✓ Microplate reader
- ✓ Assorted glassware for the preparation of reagents and buffer solutions
- ✓ Timer

Precautions for Use

- Precausion

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 de-ionized water (1 part buffer concentrate, 4 parts dH_2O).
- Wash Solution Concentrate

  The Wash Solution supplied is a 20X concentrate and must be diluted 1/20 with distilled or de-ionized water. Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35℃ before dilution can dissolve crystals.
- Enzyme-Antibody Conjugate Concentrate

  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.
- Microtiter 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 with desiccant and re-seal.
- Chicken AGP Calibrator

  The calibrator is now at a concentration of 44.20 µg/mL. Prepare the Chicken AGP Calibrators immediately prior to use according to the table below. Mix well between each step. Avoid foaming.

Standard	ng/ml	Volume added to 1x Diluent	Volume of 1x Diluent
6	200	4 µL Chicken AGP Calibrator	880 µl
5	100	300 µl standard 6	300 µl
4	50	300 µl standard 5	300 µl
3	25	300 µl standard 4	300 µl
2	12.5	300 µl standard 3	300 µl
1	6.25	300 µl standard 2	300 µl
0	0		600 µl

Sample Preparation

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. 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℃. Avoid repeated freeze-thaw cycles.

- Dilution of Samples

The assay for quantification of AGP in samples requires that each test sample be diluted before use. For a single step determination a dilution of 1/20,000 is appropriate for most serum/plasma samples. For absolute quantification of samples that yield results outside the range of the calibration curve, a lesser or greater dilution might be required.

To prepare a 1/20,000 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 5 µL, to 995 µL of 1X diluent. You now have a 1/20,000 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) into duplicate
   Standard 1 (6.25 ng/mL) into duplicate
   Standard 2 (12.5 ng/mL) into duplicate
   Standard 3 (25 ng/mL) into duplicate
   Standard 4 (50 ng/mL) into duplicate
   Standard 5 (100 ng/mL) into duplicate
   Standard 6 (200 ng/mL) into duplicate
3. Pipette 100 µL of sample (in duplicate) into pre-designated wells.
4. Incubate the Micro titer Plate at room temperature for thirty (30 ± 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 diluted Wash Solution, invert the plate and pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual Wash Solution. Repeat three times for a total of four washes.
7. Pipette 100 µL of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at room temperature for thirty (30 ± 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 µL 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 µL of Stop Solution to each well.
12. Determine the absorbance at 450 nm of the contents of each well. Calibrate the plate reader to

manufacturer's specification.

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 calibrators construct a calibration 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 calibration curve. Correct for sample dilution factor to arrive at AGP concentration in original sample.

Performance characteristics

- Indications of instability

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

Resources

Plate Layout

	A	B	C	D	E	F	G	H
12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								