KAMIYA BIOMEDICAL COMPANY

Pig Alpha-1 Acid Glycoprotein ELISA

For the quantitative determination of alpha-1 acid glycoprotein in pig serum or plasma.

Cat. No. KT-695

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT
The **K-*ASSAY*®** Pig Alpha-1 Acid Glycoprotein ELISA is an enzyme immunoassay for the quantitative determination of alpha-1 acid glycoprotein (α-1-AGP) in pig serum or plasma. For research use only.

INTRODUCTION
α-1-AGP is a positive acute phase protein in most species. However, recently it was reported that it acts as a negative acute phase reactant in pigs. In contrast, others have reported elevated levels during inflammatory diseases. α-1-AGP concentrations in pigs are high at birth but at 20 weeks of age or greater levels plateau at approximately 0.34 mg/mL. Depending on the disease or pathology being investigated, α-1-AGP may find use as a biomarker.

PRINCIPLE
The **K-*ASSAY*®** Pig α-1-AGP ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses affinity purified anti-pig α-1-AGP antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-pig 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.

COMPONENTS
- Anti-pig α-1-AGP antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8)
- Enzyme Conjugate Reagent, 11 mL
- Pig α-1-AGP Calibrator (lyophilized)
- Diluent (5X), 60 mL
- Wash Solution (20X), 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 de-ionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Micro-Plate incubator/shaker mixing speed of ~150 rpm
- Plate washer
- A microtiter plate reader capable of measuring absorbance at 450 nm, with a bandwidth of 10 nm or less and an OD range of 0-4 OD
- Graph paper (PC graphing software is optional)

GENERAL INSTRUCTIONS
All reagents should be allowed to reach room temperature (18-25℃) before use.

Serum or plasma samples should be diluted ~10,000 fold with 1X diluent in order to obtain values within the calibration range.

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 de-ionized water. The diluted wash solution is stable for 1 day at room temperature.

DILUENT PREPARATION

The dilution buffer is provided as a 5X stock. Determine the volume of 1X dilution buffer required and dilute the appropriate volume of 5X dilution buffer with 4 volumes of distilled or de-ionized water. The 1X diluent is stable for 1 day at room temperature.

CALIBRATOR PREPARATION

1. The Pig α-1-AGP calibrator 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.
2. Label 8 polypropylene or glass tubes as 200, 100, 50, 25, 12.5, 6.25, 3.13 and 0 ng/mL.
3. Into the tube labeled 200 ng/mL, prepare the 200 ng/mL working calibrator as detailed on the stock vial label.
4. Dispense 250 µL of 1X diluent into the remaining tubes.
5. Prepare the 100 ng/mL calibrator by diluting and mixing 250 µL of the 200 ng/mL calibrator with 250 µL of diluent in the tube labeled 100 ng/mL.
6. Similarly prepare the 50, 25, 12.5, 6.25 and 3.13 ng/mL calibrators by serial dilution.

Please Note: The reconstituted calibrator remains stable for at least 7 days at 4°C but should be aliquoted and stored frozen at -20°C if future use in intended.

SAMPLE PREPARATION

General Note: In order to obtain values within the range of the calibration curve we suggest that samples initially be diluted 10,000 fold using the following procedure for each sample to be tested:

1. Dispense 495 µL of 1X diluent into two tubes.
2. Pipette and mix 5 µL of the serum/plasma sample into the first tube containing 495 µL of diluent. This provides a 100 fold diluted sample.
3. Mix 5 µL of the 100 fold diluted sample with the 495 µL of diluent in the second tube. This provides a 10,000 fold dilution of the sample.
4. Repeat this procedure for each sample to be tested.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µL of calibrators and samples into the wells (we recommend that samples be tested in duplicate).
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes.
4. Remove the incubation mixture by using a plate washer or by flicking plate contents into an appropriate Bio-waste container.
5. Wash and empty the microtiter wells 5 times with 1X wash solution. This may be performed using either a plate washer (400 µL/well) or with a squirt bottle. The entire wash procedure should be performed as quickly as possible.
6. Strike the wells sharply onto adsorbent paper or paper towels to remove all residual droplets.
7. Add 100 µL of enzyme conjugate reagent into each well.
8. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes.
9. Wash as detailed in 4 and 5 above.
10. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
11. Dispense 100 µL of TMB Reagent into each well.
12. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 20 minutes.
13. Stop the reaction by adding 100 µL of Stop Solution to each well.
14. Gently mix. *It is important to make sure that all the blue color changes to yellow.*
15. Read the optical density at 450 nm with a microtiter plate reader within 15 minutes.

CALCULATION OF RESULTS

1. Calculate the average absorbance values (A_{450}) for each set of reference calibrators, and samples.
2. Construct a calibration curve by plotting the mean absorbance obtained from each reference calibrator 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.

3. Using the mean absorbance value for each sample, determine the corresponding concentration of α-1-AGP in ng/mL from the calibration curve.
4. Multiply the derived concentrations by the dilution factor to determine the actual concentration of α-1-AGP in the serum/plasma sample.
5. If available, PC graphing software may be used for the above steps. We recommend fitting to either a second order polynomial or a two-site binding equation.
6. If the OD_{450} values of samples fall outside the calibration curve when tested at the suggested dilution of 10,000, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A typical calibration curve with optical density readings at 450 nm 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 calibration curve in each experiment.

α-1-AGP (ng/mL)	Absorbance (450 nm)
200	2.694
100	2.071
50	1.271
25	0.889
12.5	0.587
6.25	0.430
3.13	0.343
0	0.278

STORAGE

The unused kit should be stored at 4℃ and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable until the expiration date provided that the components are stored as described above.

FOR RESEARCH USE ONLY

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