PIG ALPHA-1-ACID GLYCOPROTEIN (AGP) ELISA
Life Diagnostics, Inc., Catalog Number: AGP-9

INTRODUCTION

AGP is a positive acute phase protein in most species. However, Heegard et al., reported that AGP behaves as a negative acute phase reactant in pigs.[1,2] In contrast, Itoh et al., found elevated levels during inflammatory diseases (ref 3).

PRINCIPLE OF THE ASSAY

The assay uses affinity purified pig AGP antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated pig AGP antibodies for detection. Standards and diluted samples are incubated in the microtiter wells for 45 minutes. The wells are subsequently washed. HRP conjugate is added and incubated for 45 minutes. This results in AGP molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-conjugate and TMB is added and incubated for 20 minutes. If AGP is present a blue color develops. Color development is stopped by the addition of Stop solution, changing the color to yellow. Absorbance is measured at 450 nm. The concentration of AGP is proportional to absorbance and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- AGP antibody coated 96-well plate (12 x 8-well strips)
- HRP Conjugate, 11 ml
- AGP stock (lyophilized)
- 20x Wash solution; TBS50-20, 50 ml
- 5x Diluent: PAGPD60-1, 60 ml
- TMB: TMB11-1, 11 ml
- Stop solution: SS11-1, 11 ml

Materials required but not provided:

- Pipettors and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm.
- Curve fitting software

STORAGE

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

GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature before use.
2. Reliable and reproducible results will be obtained when the assay is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
4. Laboratory temperature will influence absorbance readings. Our ELISA kits are calibrated using shaking incubators set at 150 rpm and 25°C. Performance of the assay at lower temperatures will result in lower absorbance values.

DILUENT PREPARATION

The diluent is provided as a 5x stock. Prior to use estimate the final volume of diluent required for your assay and dilute one volume of the 5x stock with four volumes of distilled or deionized water. The 1x diluent is stable for 1 day at room temperature.

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 AGP stock is provided lyophilized. Add the volume of distilled or de-ionized water indicated on the vial label and mix gently until dissolved ***(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 7 polypropylene or glass tubes as 200, 100, 50, 25, 12.5, 6.25 and 3.13 ng/ml.
3. In the tube labeled 200 ng/ml prepare the 200 ng/ml standard as detailed on the stock vial label.
4. Dispense 250 µl of diluent into the tubes labeled 100, 50, 25, 12.5, 6.25 and 3.13 ng/ml.
5. Prepare the 100 ng/ml standard by mixing 250 µl of the 200 ng/ml standard with 250 µl of diluent in the tube labeled 100 ng/ml.
6. Similarly prepare the remaining standards by two-fold serial dilution.

SAMPLE PREPARATION

We found that AGP is present in pig serum at concentrations of 0.05 to 1 mg/ml. To obtain values within the range of the standard 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. 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.

AGP levels may vary with animal husbandry and study protocols. Therefore, please be aware that optimal serum or plasma dilutions should be determined empirically.

ASSAY PROCEDURE

1. Secure the desired number of 8-well strips in the holder. Unused strips should be stored in the re-sealed bag with desiccant at 4°C for future use.
2. Dispense 100 µl of standards and samples into the wells (we recommend that standards and samples be run in duplicate).
3. Incubate on an orbital micro-plate shaker at 150 rpm and 25°C for 45 minutes.

Life Diagnostics, Inc., PO Box 5205, West Chester, PA 19380
610-431-7707 – 610-431-7818 (Fax)
info@lifediagnostics.com – www.lifediagnostics.com

4. Empty and wash the microtiter wells 5x with 1x wash solution using a plate washer (400 µl/well).
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
6. Add 100 µl of HRP-conjugate into each well.
7. Incubate on a plate shaker at 150 rpm and 25°C for 45 minutes.
8. Wash as detailed above.
9. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
10. Dispense 100 µl of TMB into each well.
11. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
12. After 20-minutes, stop the reaction by adding 100 µl of Stop solution to each well.
13. Gently mix. It is important to make sure that all the blue color changes to yellow.
14. Read absorbance at 450 nm with a plate reader within 5 minutes.

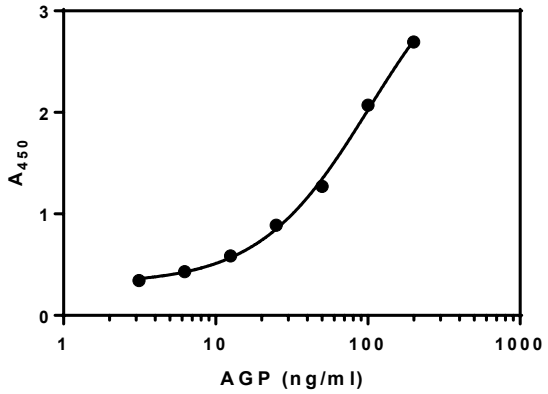
CALCULATION OF RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus $\log_{10}$ of the concentration.
2. Fit the standard curve to a four-parameter logistic regression (4PL) equation (x axis = $\log_{10}$ concentration) and determine the concentration of the samples from the standard curve (remember to derive the concentration from the antilog).
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the serum or plasma sample.
4. If the A_{450} values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve is shown below. This curve is for illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and standard curve in each experiment.

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



REFERENCES

1. Heegard PMH. et al. Pig α1-acid glycoprotein: characterization and first description in any species as a negative acute phase reactant. PLoS ONE 8(7): e68110 (2013)
2. Lv Y. Zhang X, Sun Y and Zhang S. Activation of NF-kB contributes to production of pig-major acute protein and serum amyloid A in pigs experimentally infected with porcine circovirus type 2. Res Vet Sci. S0034-5288(13)00288-9 (2013)
3. Itoh H. et al. The influence of age and health status on the serum alpha1-acid glycoprotein level of conventional and specific pathogen-free pigs. Can J Vet Res. 57:74-78 (1992)

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For technical assistance please email us at
techsupport@lifediagnostics.com