COW ALPHA-1-ACID GLYCOPROTEIN (AGP) ELISA
Life Diagnostics, Inc., Catalog Number: AGP-11

INTRODUCTION

AGP is a heavily glycosylated acute phase protein that is elevated in cow serum and milk due to infection and disease.[1] It can be used as a biomarker to evaluate health status. Studies at Life Diagnostics, Inc. (LDI) demonstrated that normal milk levels of AGP are 2.6±23.6 μg/ml (mean±SD, n=20). Levels of 449±1131 μg/ml (mean±SD, n=20) were found in milk from cows with mastitis. AGP levels in normal serum were 392±142 μg/ml (mean±SD, n=3).

PRINCIPLE OF THE ASSAY

The assay uses affinity purified cow AGP antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated cow 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, and 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 stock
- AGP stock (lyophilized)
- 20x Wash solution: TBS50-20, 50 ml
- Diluent: CSD50-1, 2 x 50 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 HRP conjugate stock should be stored at or below -20°C. The remainder of the kit should be stored at 4°C. The microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable for six months from the date of purchase provided that the components are stored as described.

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.

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 100, 50, 25, 12.5, 6.25, 3.13 and 1.56 ng/ml.
3. In the tube labeled 100 ng/ml prepare the 100 ng/ml standard as detailed on the stock vial label.
4. Dispense 250 μl of diluent into the tubes labeled 50, 25, 12.5, 6.25, 3.13 and 1.56 ng/ml.
5. Prepare the 50 ng/ml standard by mixing 250 μl of the 100 ng/ml standard with 250 μl of diluent in the tube labeled 50 ng/ml.
6. Similarly prepare the remaining standards by two-fold serial dilution.

SAMPLE PREPARATION

Serum and plasma: In studies at LDI we found AGP levels of approximately 0.4 mg/ml in cow serum. To obtain values within the range of the standard curve we suggest that serum and plasma samples be diluted 20,000-fold initially.

Milk: We found AGP levels of approximately 3 μg/ml in normal milk and measured samples after a 50-fold dilution. In milk from cows with mastitis we fond levels ranging from 3 to 5066 μg/ml. Optimal dilutions of mastitis milk must therefore be determined empirically. We suggest initially testing each mastitis sample at dilutions of 1000, 5000 and 25,000-fold.

HRP CONJUGATE PREPARATION

The anti-cow A1AGP HRP conjugate is provided as a concentrated stock. Shortly before use, dilute the stock with diluent YD50-1 as described on the stock vial label.

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.

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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.

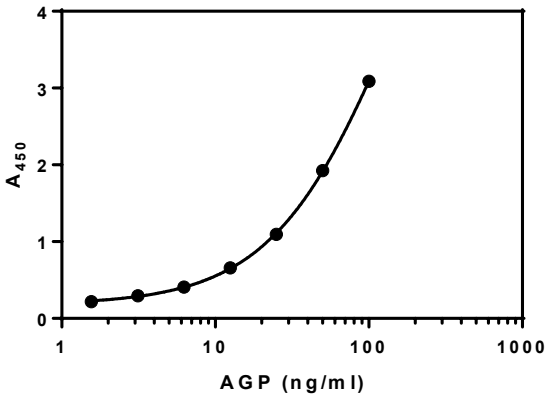
CALCULATION OF RESULTS

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)
100	3.087
50	1.924
25	1.095
12.5	0.659
6.25	0.409
3.13	0.296
1.56	0.218



REFERENCES

1. Hisaeda K, et.al. Changes in acute-phase proteins and cytokines in serum and milk whey from dairy cows with naturally occurring peracute mastitis caused by Kleibsiella pneumonia and the relationship to clinical outcome. J. Vet. Med. Sci. 73(11):1399-1404 (2011)

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For technical assistance please email us at
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