

CAT ALPHA-1-ACID GLYCOPROTEIN (AGP) ELISA

Life Diagnostics, Inc., Catalog Number: AGP-8

INTRODUCTION

AGP is an acute phase protein that is elevated in cat serum due to injury, infection and disease.¹⁻⁴ Kajikawa, et al. reported a 19-fold increase of AGP in serum from hospitalised cats and a 5.7-fold increase after injection of normal cats with lipopolysaccharide.¹ Of the positive acute phase proteins investigated (SAA, AGP, haptoglobin and CRP), they reported that AGP was the most responsive. More recently, Paltrinieri et al., reported AGP to be a powerful discriminating biomarker for diagnosis of feline infectious peritonitis.²

PRINCIPLE OF THE ASSAY

The assay uses affinity purified cat AGP antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated cat 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, 11 ml
- AGP stock (lyophilized)
- 20x Wash solution; TBS50-20, 50 ml
- 10x Diluent; YD25-10, 25 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 10x stock. Prior to use estimate the final volume of diluent required for your assay and dilute one volume of the 10x stock with nine volumes of distilled or deionized water.

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 5 polypropylene or glass tubes as 50, 25, 12.5, 6.25 and 3.13 ng/ml.
3. In the tube labeled 50 ng/ml prepare the 500 ng/ml standard as detailed on the stock vial label.
4. Dispense 250 µl of diluent into the tubes labeled 25, 12.5, 6.25 and 3.13 ng/ml.
5. Prepare the 25 ng/ml standard by mixing 250 µl of the 50 ng/ml standard with 250 µl of diluent in the tube labeled 25 ng/ml.
6. Similarly prepare the remaining standards by two-fold serial dilution.

SAMPLE PREPARATION

We observed AGP concentrations of approximately 0.15 mg/ml in serum of healthy American short haired cats. However, in serum from diseased cats, concentrations can reach 5 mg/ml or greater.¹⁻⁴ 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.

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.

To avoid matrix effects, do not test samples at dilutions below 4000-fold.

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.
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.

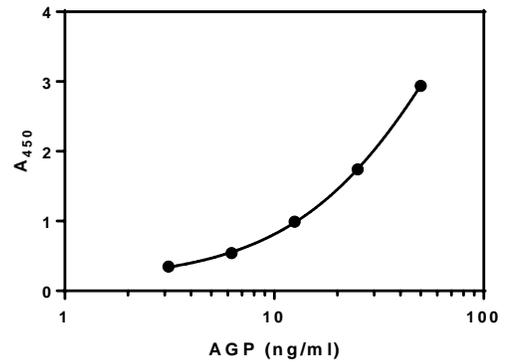
CALCULATION OF RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus log₁₀ of the concentration.
2. Fit the standard curve to a four-parameter logistic regression (4PL) equation (x axis = log₁₀ 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 A₄₅₀ 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)
50	2.940
25	1.741
12.5	0.991
6.25	0.540
3.13	0.347



ASSAY CHARACTERISTICS

Typical results obtained with serum from five American short-haired cats are shown in the table below. Samples were diluted as indicated and concentrations (ng/ml) determined. Serum concentrations (µg/ml) were calculated by multiplying the concentrations of the samples by their respective dilution factors. Average values, standard deviation (SD) and coefficient of variation (CV) were then calculated.

Sample	Dilution	A450	ng/ml	ug/ml	Average ug/ml	SD (ug/ml)	CV
2325	4000	1.637	31.51	126.1	137.5	10.4	7.5
	8000	0.900	16.51	132.1			
	16000	0.526	8.90	142.4			
	32000	0.318	4.67	149.3			
2326	4000	2.086	40.65	162.6	160.7	8.6	5.4
	8000	1.016	18.87	151.0			
	16000	0.573	9.86	157.7			
	32000	0.352	5.36	171.4			
2327	4000	1.931	37.50	150.0	139.7	12.0	8.6
	8000	0.850	15.49	124.0			
	16000	0.509	8.55	136.8			
	32000	0.316	4.62	148.0			
2328	4000	1.951	37.91	151.6	147.7	5.9	4.0
	8000	0.964	17.81	142.5			
	16000	0.527	8.92	142.7			
	32000	0.325	4.81	153.8			
2329	4000	3.421	67.83	271.3	276.2	18.6	6.7
	8000	1.950	37.88	303.1			
	16000	0.919	16.90	270.4			
	32000	0.488	8.13	260.0			

REFERENCES

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