Canine Alpha-1 Acid Glycoprotein

Catalog No. IRKTAH1111

Introduction

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.

Assay Principle

The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the AGP present in samples reacts with the anti-AGP antibodies which have been adsorbed to the surface of polystyrene microtitre wells. After the removal of unbound proteins by washing, anti-AGP antibodies conjugated with horseradish peroxidase (HRP), are added. These enzyme-labeled antibodies form complexes 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 varies directly with 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 standard curve constructed from the standards, and corrected for sample dilution.

Reagents Provided

DILUENT CONCENTRATE (Running Buffer) One bottle containing 50 ml of a 5X concentrated diluent running buffer.

WASH SOLUTION CONCENTRATE One bottle containing 50 ml of a 20X concentrated wash solution.

ENZYME-ANTIBODY CONJUGATE 100x One vial containing 150 µL of affinity purified anti-Dog AGP antibody conjugated with horseradish peroxidase in a stabilizing buffer.

CHROMOGEN-SUBSTRATE SOLUTION One vial containing 12 mL of 3,3',5,5'- tetramethybenzidine (TMB) and hydrogen peroxide in citric acid buffer at pH 3.3.

STOP SOLUTION One vial containing 12 ml 0.3 M sulfuric acid.

ANTI-DOG AGP ELISA MICRO PLATE Twelve removable eight (8) well micro well strips in well holder frame. Each well is coated with affinity purified anti-Dog AGP.

DOG AGP CALIBRATOR Two vials containing Dog AGP calibrator.

Reagent Preperation

DILUENT CONCENTRATE The Diluent Solution supplied is a 5X Concentrate and must be diluted 1/5 with distilled or deionized water (1 part buffer concentrate, 4 parts dH2O).

WASH SOLUTION CONCENTRATE The Wash Solution supplied is a 20X Concentrate and must be diluted 1/20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH2O). Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals.

ENZYME-ANTIBODY CONJUGATE Calculate the required amount of working conjugate solution for each microtitre 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.

ANTI-DOG AGP ELISA MICRO 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 and re-seal.

DOG AGP CALIBRATOR Add 1.0 ml of distilled or de-ionized water to one of the Dog AGP calibrator and mix gently until dissolved. The calibrator is now at a concentration of 2.25µg/ml(the reconstituted calibrator should be aliquoted and frozen if future use is intended). Dog C3 standards need to be prepared immediately prior to use (see chart below). Mix well between each step. Avoid foaming.

Storage and Stability

DILUENT The 5X Diluent Concentrate is stable until the expira- tion 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°C.

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°C) or at 4-8°C.

ENZYME-ANTIBODY CONJUGATE Undiluted horseradish peroxidase anti-AGP conjugate should be stored at 4-8°C and diluted immediately prior to use. The working conjugate solution is stable for up to 8 hours.

CHROMOGEN-SUBSTRATE SOLUTION The Substrate Solution should be stored at 4-8°C and is stable until the expiration date.

STOP SOLUTION The Stop Solution should be stored at 4-8°C and is stable until the expiration date.

ANTI-DOG AGPELISA MICRO PLATE Anti-Dog AGP coated wells are stable until the expiration date, and should be stored at 4-8°C in sealed foil pouch with desiccant pack.

DOG AGP CALIBRATOR The lyophilized Dog AGP calibrator should be stored at 4C or frozen until reconstituted. The reconstituted calibrator should be aliquoted out and stored frozen

Collection and Handling

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. Care should be taken to minimize hemolysis, excessive hemolysis can impact your results. Assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze- thaw cycles.

Precautions 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.

Required Material

- Precision pipette (2 µL to 200 µL) for making and dispensing dilutions
- Test tubes
- Microtitre washer/aspirator
- Distilled or Deionized H2O
- Microtitre Plate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer
- Vortex mixer

Assay Procedure

The assay for quantification of AGP in samples requires that each test sample be diluted before use. For a single step determination a dilution at 1/5,000 is appropriate for most serum/plasma samples. For absolute quantification, samples that yield results outside the range of the standard curve, a lesser or greater dilution might be required.

To prepare a 1/5,000 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 10 µL, to 490 µL of 1X diluent. You now have a 1/5,000 dilution of your sample. Mix thoroughly at each stage.

1. Bring all reagents to room temperature before use.
2. Pipette 100 µL of:

   Standard 0 (0.0 ng/ml) in duplicate
   Standard 1 (6.17 ng/ml) in duplicate
   Standard 2 (18.5 ng/ml) in duplicate
   Standard 3 (55.6 ng/ml) in duplicate
   Standard 4 (166.7 ng/ml) in duplicate
   Standard 5 (500 ng/ml) in duplicate
   Standard 6 (1500 ng/ml) in duplicate

3. Pipette 100 µL of sample (in duplicate) into pre designated wells.
4. Incubate the micro titer plate at room temperature for sixty (60 ± 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 wash buffer, invert the plate then pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 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 twenty (20 ± 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 (450 nm) of the contents of each well. Calibrate the plate reader to air.

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.

RESULTS

1. Subtract the average background value from the test values for each sample.
2. Using the results observed for the standards construct a Standard 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 standard curve. Correct for sera dilution factor to arrive at the AGP concentration in original samples