ab291068- Cat AGP ELISA Kit

For Determination of Alpha 1-Acid Glycoprotein in Cat Samples.
For research use only and is not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab291068

Storage and Stability

On receipt, entire assay kit should be stored at 4°C, protected from light. The expiration date for the kit and its components is stated on the box label. All components should be stable up to the expiration date if stored and used per this kit protocol insert.

Materials Supplied

Item	Quantity	Storage Condition
ELISA Micro Plate, antibody coated	8 x 12 unit	4-8°C
Enzyme Conjugated Detection Antibody	150 μL	4-8°C in the dark
Calibrator	1 vial	4-8°C
5X Diluent Concentrate	50 mL	4-8°C
20X Wash Solution Concentrate	50 mL	4-8°C
Chromogen Substrate Solution	12 mL	4-8°C in the dark
STOP Solution	12 mL	4-8°C

Materials Required, Not Supplied

These material are not included in the kit but will be required to successfully utilize this assay:

- Precision pipettes (2 μL to 100 μL) for making and dispensing dilutions
- Test tubes
- Squirt bottle or Microtitre washer/aspirator
- Distilled or Deionized H_2O
- Microtitre Plate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Centrifuge for sample collection
- Anticoagulant for plasma collection
- Timer

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.
- Bring all reagents to room temperature (16°C to 25°C) before use.

Diluent Concentrate: Dilute 1:5 with distilled or deionized water (1 part buffer concentrate, 4 parts dH_2O).

Wash Solution Concentrate: Dilute 1:20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH_2O). Crystal formation in the concentrate may occur 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. Dilute immediately before use and protect from light. Mix uniformly, but gently. Avoid foaming.

Pre-coated ELISA Micro Plate: Ready to use as supplied. Unseal foil 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 along with desiccant.

Cat AGP Calibrator: **The calibrator is provided at the concentration stated on the vial.** Reconstitute the Cat AGP Calibrator with distilled or de-ionized water as specified on the vial and mix gently until dissolved. The amount of calibrator is shown on the vial, and after reconstitution will have a concentration of X μg/mL, where X is the amount on the vial (the reconstituted calibrator should be aliquoted and stored frozen if future use is intended).

Sample preparation
Sample Preparation and Handling

- All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions when handling and disposing.
- If blood samples are clotted, grossly hemolyzed, lipemic, or the integrity of the sample is of concern, make a note and interpret results with caution.
- The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. Remove serum and assay immediately or aliquot and store samples at –80°C (preferably) or -20°C. Avoid repeated freeze-thaw cycles.

Plasma: Blood should be collected into a container with an anticoagulant and then centrifuged. Assay immediately or aliquot and store samples at –80°C (preferably) or -20°C. Avoid repeated freeze-thaw cycles.

Urine: Collect mid-stream using sterile or clean urine collector. Centrifuge to remove cell debris. Assay immediately or aliquot and store samples at –80°C (preferably) or -20°C. Avoid repeated freeze-thaw cycles.

Known interfering substances - Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.

Sample Dilution

– The assay requires that each test sample be diluted before use. All samples should be assayed in duplicate each time the assay is performed. The recommended dilutions are only suggestions. Dilutions should be based on the expected concentration of the unknown sample such that the diluted sample falls within the dynamic range of the standard curve. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

Serum: Recommended starting dilution is 1:10,000. To prepare a 1:10,000 dilution of a sample, transfer 5 µL of sample to 495 µL of 1X diluent. This gives you a 1:100 dilution. Next, dilute the 1:100 by transferring 5 µL into 495 µL of 1X diluent. This gives you a 1:10,000 dilution. Mix thoroughly each stage.

Plasma: Recommended starting dilution is 1:10,000. To prepare a 1:10,000 dilution of a sample, transfer 5 µL of sample to 495 µL of 1X diluent. This gives you a 1:100 dilution. Next, dilute the 1:100 by transferring 5 µL into 495 µL of 1X diluent. This gives you a 1:10,000 dilution. Mix thoroughly each stage.

Assay Protocol
- All standards and samples should be run at least in duplicate.

Calibrator preparation :
Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.

1. Add 1.0 mL of distilled or de-ionized water to the Cat AGP Calibrator and mix gently until dissolved. The calibrator is now at the concentration stated on the vial.

   Note: The reconstituted Cat AGP Calibrator should be aliquoted and stored frozen. Avoid multiple freeze-thaw cycles.
2. Label tube numbers 1 - 7.
3. Prepare Standard #1 by adding the appropriate volume of 1X Diluent Solution (see below) to tube #1. Add 80 µL of stock Cat AGP Calibrator to obtain a concentration at 100 ng/mL and mix thoroughly and gently.

***Example: Note: This example is for demonstration purposes only. Please remember to check your calibrator vial for the actual concentration of calibrator provided**.

CS = Starting concentration of reconstituted Cat AGP Calibrator
CF = Final concentration of Cat AGP Calibrator for the assay procedure (100 ng/mL)
VA = Total volume of stock Cat AGP Calibrator to dilute (e.g. 80 µL)
VD = Total volume of 1X Diluent Solution required to dilute stock Cat AGP Calibrator to prepare Standard #1
VT = Total volume of Standard #1
DF = Dilution factor

Calculate the dilution factor (DF) between stock calibrator & the Standard #1 final conc.:

CS/CF = DF
1225 /100 = 12.25

Calculate the final volume VD required to prepare the Standard #1 at 100 ng/mL

VA * DF = VT
VD = VT - VA
80 * 12.25= 980 µL
VD = 980 – 80 = 900 µL
To tube #1, add 80 µL of reconstituted Cat AGP Calibrator to 900 µL of 1X Diluent Solution to obtain a concentration at 100 ng/mL (Standard #1).

4. Add 300 µL 1X Diluent Solution into tube numbers 2 - 6.
5. Prepare Standard #2 by adding 300 µL Standard #1 to tube #2. Mix thoroughly and gently.
6. Prepare Standard #3 by adding 300 µL from Standard #2 to #3. Mix thoroughly and gently.
7. Using the table below as a guide to prepare further serial dilutions. 1X Diluent Solution serves as the zero standard (0 ng/mL)

Standard #	Volume to Dilute (µL)	Diluent (µL)	Total Volume (µL)	Starting Conc (ng/mL)	Final Conc. (ng/mL)
7	See step 3				100
6	300	300	600	100	50
5	300	300	600	50	25
4	300	300	600	25	12.50
3	300	300	600	12.50	6.25
2	300	300	600	6.25	3.13
1	300	300	600	3.13	1.57

The Standards and the test sample(s) should be loaded into the ELISA wells as quickly as possible to avoid a shift in OD readings. Using a multichannel pipette would reduce this occurrence.

8. Pipette 100 µL of sample (in duplicate) into pre-designated wells.
9. Incubate the micro titer plate at room temperature for sixty (60 ± 2) minutes. Keep plate covered and level during incubation.
10. Following incubation, aspirate the contents of the well.
11. 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.
12. Pipette 100 µL of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at room temperature for thirty (30 ± 2) minutes. Keep plate covered in the dark and level during incubation.
13. Wash and blot the wells as described in Steps 5/6.
14. Pipette 100 µL of TMB Substrate Solution into each well.
15. Incubate in the dark at room temperature for precisely ten (10) minutes.
16. After ten minutes, add 100 µL of Stop Solution to each well.
17. Determine the absorbance (450 nm) of the contents of each well within 30 minutes. Calibrate the plate reader to manufacturer's specifications.

Calculation
- Subtract the average background value (Average absorbance reading of Standard zero) from the test values for each sample.
- Average the duplicate readings for each standard and use the results to construct a Standard Curve. Construct the standard curve by reducing the data using computer software capable of generating a four parameter logistic curve fit. A second order polynomial (quadratic) or other curve fits may also be used; however, they will be a less precise fit of the data.

- Interpolate test sample values from standard curve. Correct for sera dilution factor to arrive at the AGP concentration in original samples.