Feline Alpha-1-Acid Glycoprotein (fAGP) ELISA

Product Number: RL0006

Assay Precautions

- Do not use kit or individual reagents past the expiry date.
- Do not mix or substitute reagents from different kit batches.
- Samples should be stored refrigerated or frozen if they are not to be analysed immediately after collection.
- Avoid repeat freeze thawing of samples.
- Where possible avoid the use of haemolysed or lipemic serum.
- Standards and samples are recommended to run in duplicate.
- Reagents should be mixed (avoiding foaming) and allowed to come to room temperature before use.
- Avoid leaving reagents in direct sunlight and/or above 4ºC for prolonged periods.
- Cover or cap all reagents when not in use.
- Use clean, preferably disposable labware for all reagent preparation.
- Care must be taken not to contaminate reagents. Use fresh tips for each sample and reagent.
- Care should be taken when dispensing reagents into the wells not to scratch the bottom/side of the well with the tip.
- Liquid should be dispensed down the side of the well.
- Do not allow wells to dry out during the procedure and never insert absorbent tissue into the wells.
- The bottom outer surface of the well should be clean and dry before reading.
- Absorbance should be read within 15 minutes of adding stop solution.

Safety Precautions

- For *in vitro* research purposes only.
- Dispose of all clinical samples, infected or potentially infectious material in accordance with good laboratory practice.
- Wear disposable gloves and safety glasses where appropriate.
- The kit contains reagents that may cause irritation to skin and eye. Any reagent which comes into contact with the skin or eye should be washed off with water immediately.
- The kit contains reagents that may cause irritation to respiratory or gastrointestinal tract if inhaled or ingested. Seek medical attention if you feel unwell.

Feline Alpha-1-Acid Glycoprotein (fAGP) ELISA

Product Number: RL0006

Intended Use

The feline alpha-1-acid glycoprotein (AGP) enzyme-linked immunosorbent assay (ELISA) is intended for the quantitative measurement of AGP in feline serum.

Clinical Use

Acute phase proteins (APPs) such as AGP are serum proteins that increase in concentration 24 to 48 hours following infection, inflammation, or trauma. The circulating concentrations of these proteins can provide an objective measure of the health status of an animal and are increasingly being used as markers of animal health and welfare.

Serum concentrations of APPs are related to the severity of the underlying condition, and provide a ready means of evaluating both the presence and extent of disease.

In a healthy animal, AGP is present in very low levels ranging from less than or equal to 0.5 mg/mL in cats.

Methodology

AGP in a sample of feline serum is captured by an anti-feline AGP antibody that has been immobilised on the surface of wells. Captured AGP is detected by anti-feline AGP antibody which has been conjugated to horseradish peroxidase (HRP).

With the addition of TMB substrate a blue colour develops. Upon stopping the reaction, a yellow colour is obtained in proportion to the amount of AGP in the sample which may be measured at a wavelength of 450 nm.

By comparison to supplied standards with known concentrations of AGP, the concentration of AGP in the sample can be determined.

Reagents Provided

- fAGP antibody coated wells
  - 1 x 96 well plate
- Wash buffer (20x)
  - 30 mL
- Assay diluent
  - 30 mL
- fAGP standard
  - 2 x 1.25 mL
- Low and high matrix controls
  - 1 x 50 µL of each
- Anti-fAGP conjugate
  - 1 x 15 mL
- Substrate
  - 1 x 15 mL
- Stop solution
  - 1 x 15 mL

Additional Materials Required

- Plate reader capable of measurement at 450 nm and 655 nm.
- A variety of micropipettes and disposable tips capable of dispensing 5 µL – 1000 µL.
- A multichannel pipette capable of dispensing 100 µL (optional).
- Microcentrifuge tubes.
- Distilled water.
- Plate washer (optional).
- Absorbent paper towels.
- 96 well plate cover/sealer.

Sample Preparation

The serum should be separated from the red blood cells as soon as possible after collection. Samples should be frozen if analysis cannot be performed immediately.

It is recommended that serum samples should be diluted 1 in 75,000 in assay diluent prior to testing.

It may be necessary to determine the optimal dilution empirically; however we do not recommend diluting samples more than 1 in 200,000.

Feline Alpha-1-Acid Glycoprotein (fAGP) ELISA

Product Number: RL0006

Storage and Stability

The contents of the feline AGP ELISA kit should be stored at 2-8°C and used within the expiry date detailed on the packaging.

Individual components are labelled with individual expiry dates; however the kit should be used within expiry date detailed on the packaging.

Reagent Preparation

- fAGP antibody coated wells
  - Ready to use
- Wash buffer (20x)
  - Ensure that any crystals that may have formed in the wash buffer have completely dissolved prior to dilution.
  - Dilute 1 in 20 in distilled water prior to use.
- Assay Diluent
  - Ready to use
- fAGP standard
  - The fAGP standard should be diluted in assay diluent as detailed below

1. Label 8 tubes S1-S8. Add 250 µL assay diluent to tubes S2-S8.
2. Invert fAGP standard several times before use and add 250 µL to tube S1.
3. Add 250 µL fAGP standard to tube S2. Mix well and serially dilute as directed in table 1.

Table 1: Preparation of Standard Curve

Standard tube number	fAGP concentration (ng/mL)	Volume of fAGP Standard (µL)	Volume of assay diluent (µL)	Serial Dilution
S1	80	250	-	-
S2	40	250	250	-
S3	20	-	250	250µL of S2
S4	10	-	250	250µL of S3
S5	5	-	250	250µL of S4
S6	2.5	-	250	250µL of S5
S7	1.25	-	250	250µL of S6
S8	0	-	250	-

- Anti-fAGP conjugate
  - Ready to use
- Substrate
  - Ready to use

All reagents should be warmed to room temperature before use.

Assay Procedure

1. Prepare appropriate volume of wash buffer (1x) as described in “Reagent Preparation”.
2. Dilute serum 1 in 75,000 in assay diluent as detailed below – mix well.
   a) Add 5 µL of neat serum to 995 µL of assay diluent (1 in 200 dilution).
   b) Add 5 µL of 1 in 200 dilution to 1870 µL assay diluent (final dilution of 1 in 75,000).
3. Prepare the standard curve as described in “Reagent Preparation” table 1.
4. Dilute matrix controls in assay diluent as instructed on the tube label.
5. Determine the number of strips needed. Re-bag any excess strips, re-seal and store at 2-8°C. Use within expiry date stated on the kit.
6. Add 100 µL of standard, control or diluted sample per well. Cover the plate and incubate for 60 minutes at room temperature.
7. Aspirate and wash 3 times with 300 µL/ well wash buffer (1x). After the final washing, blot dry on absorbent tissue.
8. Add 100 µL anti-fAGP conjugate to each well. Cover and incubate for 60 minutes at room temperature.
9. Aspirate and wash 3 times with 300 µL/ well wash buffer (1x). After the final washing blot dry on absorbent tissue.
10. Add 100 µL substrate to each well. Cover and incubate at room temperature until S2 reads >0.55 OD at 655nm (approximately 15 minutes).
11. Add 50 µL stop solution to each well.
12. Read plates on a plate reader at 450 within 15 minutes of adding stop solution.

Interpretation

1. Calculate the mean absorbance for each standard and sample.
2. Plot the mean absorbance for each standard concentration (y-axis) against the Log_{10} AGP concentration (x-axis) using a sigmoidal 4PL fit

Feline Alpha-1-Acid Glycoprotein (fAGP) ELISA

Product Number: RL0006

3. Determine the concentration of AGP in the samples from the standard curve, multiplying the value obtained for the samples by the appropriate dilution factor.
4. Matrix controls should give AGP concentration within ranges stated on the tube label.*

Samples that give signal greater than the top standard should be further diluted and re-analysed.

**It is recommended that each laboratory should establish its own reference ranges.*

Performance Characteristics

1. Assay Range

1.25 – 80 ng/mL

2. Intra-Assay Reproducibility

Two feline pools were assayed in replicates of five on one plate to determine intra (within) assay reproducibility.

	Pool 1	Pool 2
n	5	5
Mean (ng/mL)	6.75	38.77
Standard Deviation	0.14	3.30
%CV	2.1	8.5

Mean intra-assay variation: 5.3%.

3. Inter-Assay Reproducibility

Two feline pools were assayed in replicates of five on four separate plates to determine inter-assay reproducibility.

	Pool 1	Pool 2
n	20	20
Mean (ng/mL)	6.56	37.36
Standard Deviation	1.19	6.57
%CV	18.2	17.6

Mean inter-assay variation: 17.9%.

4. Sensitivity

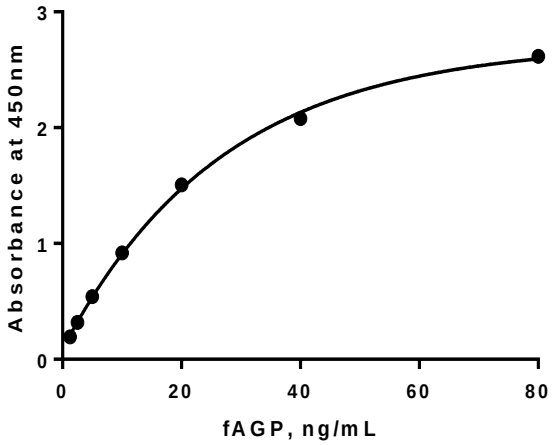
1.25 ng/mL.

5. Dilution linearity

1 in 1,563 - 1 in 200,000

Typical Standard Curve

A typical standard curve is shown below. This should not be used to determine the fAGP concentration of unknown samples. A standard curve must always be run with each assay.



Summary of Assay Procedure

100 µL of test samples and standards (S1-S8) to duplicate wells, as appropriate
Incubate 60 minutes at room temperature
Wash plate 3 times with 300 µL/ well wash buffer (1x)
Add 100 µL/ well of anti-fAGP conjugate
Incubate 60 minutes at room temperature
Wash plate 3 times with 300 µL/ well wash buffer (1x)
Add 100 µL/ well of substrate
Incubate at room temperature until S2 reaches >0.55 OD at 655 nm (approximately 15 minutes)
Add 50 µL/ well of stop solution
Read absorbance at 450 nm