KAMIYA BIOMEDICAL COMPANY

Dog Pancreatitis-Associated Protein-1 (PAP-1) ELISA

For the quantitative determination of PAP-1 in dog fecal extracts and serum

Cat. No. KT-1893

For Research Use Only.

PRODUCT INFORMATION
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PRODUCT
The ***K-ASSAY***® Dog Pancreatitis-Associated Protein-1 (PAP-1) ELISA is an enzyme immunoassay for the quantitative determination of PAP-1 in dog fecal extracts and serum. For research use only.

INTRODUCTION
Pancreatitis-associated protein-1 (PAP-1) is a 16.5 kDa protein that is expressed at high levels during inflammatory bowel disease (IBD) in humans. In our studies, we found PAP-1 levels of 9.7 ± 17.7 µg/g (mean ± SD, n=13) and 115 ± 264 µg/g (mean ± SD, n=14) in feces from healthy dogs and dogs with IBD respectively, suggesting that PAP-1 may be a biomarker of IBD in dogs.

PRINCIPLE
The assay uses two dog PAP-1 antibodies, one for solid phase immobilization and one conjugated to horseradish peroxidase (HRP), for detection. Calibrators and diluted samples (100 µL) are incubated in antibody coated microtiter wells for 45 minutes. After washing the wells, HRP-conjugate (100 µL) is added and incubated for 45 minutes. PAP-1 molecules, if present, are sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-conjugate. TMB is added and incubated for 20 minutes. If PAP-1 is present, a blue color develops. Color development is stopped by addition of stop solution, changing the color to yellow. Absorbance is measured at 450 nm. The concentration of PAP-1 is proportional to absorbance and is derived from a calibration curve.

COMPONENTS
- Anti-PAP-1 coated plate (12 x 8-well strips)
- HRP conjugate stock. Store ≤ -20°C
- PAP-1 calibrator. Store ≤ -20°C
- 20x Wash solution: 50 mL
- Diluent: 2 x 50 mL
- Fecal extraction buffer: 50 mL
- TMB: 11 mL
- Stop solution: 11 mL

MATERIALS REQUIRED BUT NOT PROVIDED
- Pipettors and tips
- Distilled or deionized water
- Polypropylene tubes
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm
- Graphing software

STORAGE
The kit should be stored at 4°C and the microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable until the expiration date shown on the box label.

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. It is important that calibrators and samples be added to the ELISA plate quickly. If testing large numbers of samples, rather than pipetting calibrators and samples from individual tubes into the ELISA plate, we recommend the following. First,

pipette an excess volume of calibrators and samples into appropriate wells of a blank polystyrene 96-well plate. Then use an 8- or 12-channel multi-pipettor to quickly transfer 100 µL aliquots to the appropriate wells of the ELISA plate.
4. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
5. Laboratory temperature will influence absorbance readings. The assay was calibrated using a shaking incubator set at 150 rpm and 25°C. Performance of the assay at lower temperatures and mixing speeds may 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 de-ionized water. Unused wash buffer may be stored at 4°C for one week.

DILUENT PREPARATION

Both the assay diluent and the fecal extraction diluent are prepared ready for use. DO NOT substitute other buffers

CALIBRATOR PREPARATION

1. The calibrator consists of dog PAP-1 lyophilized in a stabilizing matrix. Reconstitute as indicated on the vial label.
2. In a polypropylene tube labeled 20 ng/mL, prepare the 20 ng/mL calibrator as indicated on the stock vial label, using diluent for dilution.
3. Label seven polypropylene tubes as 10, 5, 2.5, 1.25, 0.625, 0.313 and 0 ng/mL. Dispense 0.25 mL of diluent into each.
4. Pipette 0.25 mL of the 20 ng/mL PAP-1 calibrator into the tube labeled 10 ng/mL and mix. This provides the 10 ng/mL PAP-1 calibrator.
5. Similarly prepare the 5 to 0.313 ng/mL calibrators by two-fold serial dilution. Although the PAP-1 stock is stable for at least one day at room temperature it should be frozen at or below -20°C for optimum stability

SAMPLE PREPARATION

Fecal Extracts

Fecal extracts can be prepared using the following procedure.
1. Weigh ≈0.1 g feces into a tared microcentrifuge tube.
2. Add 9 volumes of fecal extraction buffer (i.e., 0.10 g feces + 0.90 mL buffer)
3. Cap the tube and vortex for one minute.
4. Centrifuge in a microcentrifuge for 5 minutes at 15,000 rpm.
5. Remove and save the supernatant in a clean microcentrifuge tube. This represents a 10-fold dilution of the fecal sample.
6. Freeze the extracts at or below -20°C if they are not to be tested immediately.
7. Prior to testing the extract should be further diluted at least 100-fold with diluent (3 µL + 297 µL). Please note that this represents a 1,000- fold “dilution” of the fecal sample. In our studies, we found PAP-1 levels ranging from 0.1 µg/g of feces in healthy dogs to >1,500 µg/g of feces from dogs with IBD. We suggest that fecal extracts be tested initially at a dilution of 1,000-fold (a 100-fold dilution of the extract) but optimal dilutions must be determined empirically.

Serum

Serum can be tested after dilution in diluent. We found levels ranging from 0.005 µg/mL in serum from healthy dogs to 8 µg/mL in serum from dogs with gastrointestinal disorders. We suggest testing serum at a 100-fold dilution. Optimal dilutions must be determined empirically.

ASSAY PROCEDURE

1. Secure the desired number of 8-well strips in the cassette. Unused strips should be stored in a sealed bag with desiccant at 4°C.
2. Dispense 100 µL of calibrators and samples into the wells (we recommend that calibrators and samples be run in duplicate).
3. Incubate on a 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. Dispense 100 µL of HRP conjugate into the wells.
6. Incubate on a plate shaker at 150 rpm and 25°C for 45-minutes.
7. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
8. Dispense 100 µL of TMB into each well.
9. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
10. After 20 minutes, stop the reaction by adding 100 µL of Stop solution to each well.
11. Gently mix. It is important to make sure that all the blue color changes to yellow.
12. Read absorbance at 450 nm with a plate reader within 5 minutes.

CALCULATION OF RESULTS

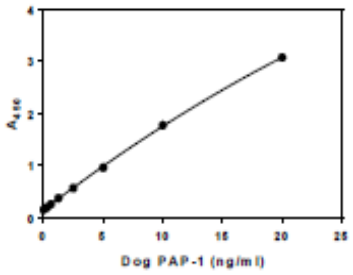
1. Using curve fitting software, construct a calibration curve by plotting absorbance values of the calibrators versus PAP-1

concentration.
2. Fit the calibration curve using graphing software. We typically use a two-site, total and nonspecific binding model.
3. Multiply the derived concentration by the dilution factor to determine the concentration in the sample.
4. If the A_{450} values of samples fall outside the calibration curve, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A typical calibration curve with absorbance at 450 nm on the Y-axis against PAP-1 concentrations on the X-axis is shown below. This curve is for illustration only.

PAP-1 (ng/ml)	A_{450}
20	3.081
10	1.776
5	0.958
2.5	0.564
1.25	0.370
0.625	0.256
0.313	0.190
0	0.148



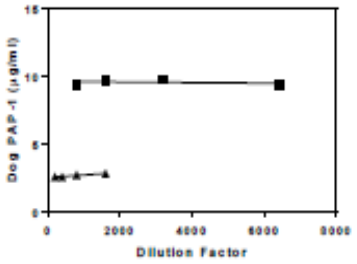
PERFORMANCE

Inter-Assay Precision (Precision between assays): Four serum samples were tested in quadruplicate in three separate assays to assess intra-assay and inter-assay precision.

Intra-Assay Precision				
Sample	1	2	3	4
Dilution	100	400	100	1600
n	4	4	4	4
Average (ng/ml)	130	2704	471	9344
Std Deviation	8	65	19	428
CV%	6.3	2.4	3.9	4.6

Inter-Assay Precision				
Sample	1	2	3	4
Dilution	100	400	100	1600
n	3	3	3	3
Average (ng/ml)	142	2715	457	9366
Std Deviation	24	295	44	741
CV%	17.2	10.9	9.6	7.9

Linearity: To assess the linearity of the assay, two serum samples containing PAP-1 at concentrations of 2.72 and 9.37 µg/mL were serially diluted to produce values within the dynamic range of the assay.



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