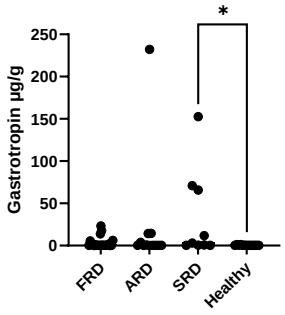
Dog Gastrotropin ELISA GTP-4

INTRODUCTION

Gastrotropin (GTP), also known as ileal fatty acid binding protein, and FABP6, is a cytoplasmic protein involved in intestinal fatty acid transport. In studies at Life Diagnostics, we found that levels were significantly elevated in fecal extracts from dogs with steroid responsive diarrhea (SRD) but were not significantly increased in fecal extracts from dogs with food responsive or antibiotic responsive diarrhea.



PRINCIPLE OF THE ASSAY

The assay uses affinity purified polyclonal antibodies against recombinant dog gastrotropin that were developed at Life Diagnostics. One is used as coating antibody. The other is conjugated to HRP and used for detection. Standards and diluted samples (100 µl) are incubated in the coated microtiter wells for 45 minutes. After washing the wells, HRP-conjugate (100 µl) is added and incubated for 45 minutes. If gastrotropin molecules are present, they are sandwiched between the capture and detection antibodies. The wells are then washed to remove unbound HRP-conjugate. TMB is added and incubated for 20 minutes. If gastrotropin is present, a blue color develops. Color development is stopped after 20-minutes by addition of Stop Solution, changing the color to yellow. Absorbance is measured at 450 nm. The concentration of gastrotropin is proportional to absorbance and is derived from a standard curve.

MATERIALS

Materials provided with the kit:

- Anti-gastrotropin coated plate (12 x 8-well strips)
- Anti-gastrotropin HRP stock
- Gastrotropin. **Store at -20°C**
- 20x Wash Solution: TBS50-20, 50 ml
- Diluent: SB50-1, 2 x 50 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 tubes or 96-well polystyrene plates
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker & washer
- TBE buffer[1]
- Plate reader capable of measuring absorbance at 450 nm
- Graphing software

STORAGE

Store the standard stock at -20°C. The remainder of the kit should be stored at 4°C and the microtiter plate should be kept in a sealed bag with desiccant. The kit 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. It is important that standards and samples be added to the ELISA plate quickly. If testing large numbers of samples, rather than pipetting standards and samples from individual tubes into the ELISA plate, we recommend the following: pipette an excess volume of standards and samples into wells of a blank polystyrene 96-well plate[2]. Then use an 8 or 12-channel multi-pipettor to quickly transfer 100 µl aliquots to the wells of the antibody-coated plate.
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. The assay was calibrated using a shaking incubator set at 150 rpm and 25°C. Performing the assay at lower temperatures and mixing speeds may result in lower absorbance values.

WASH SOLUTION

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. Unused wash buffer may be stored at 4°C for one week.

DILUENT

The Diluent (SB50-1) is formulated for measurement of gastrotropin in fecal extracts. It is supplied ready to use. DO NOT substitute other buffers.

[1] 150 mM NaCl, 10 mM Tris, 1 mM EDTA, pH 7.4

[2] Standards and sample dilutions may also be prepared directly in a blank polystyrene plate.

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STANDARD

1. The stock is lyophilized. It is comprised of recombinant dog gastrotropin in a stabilizing matrix. Reconstitute it with deionized water as described on the vial label and gently mix. Prepare the 20 ng/ml standard as described on the label.
2. Label seven polypropylene tubes as 10, 5.0, 2.5, 1.25, 0.625, 0.313 and 0 ng/ml. Dispense 0.25 ml of diluent SB50-1 into each.
3. Pipette 0.25 ml of the 20 ng/ml gastrotropin standard into the tube labeled 10 ng/ml and mix. This provides the 10 ng/ml gastrotropin standard.
4. Similarly prepare the 5.0 to 0.313 ng/ml standards by two-fold serial dilution.

IMPORTANT – If future use of the stock is intended, the sealed vial must be frozen at or below -20°C within one hour of reconstitution.

HRP CONJUGATE

The HRP conjugate is provided as a concentrated stock. Dilute the stock with SB50-1 diluent as described on the vial label 5-15 minutes before it is required.

SAMPLES

The assay is intended for measurement of gastrotropin in fecal extracts. Extracts may be prepared as follows:

1. Weigh approximately 100 mg of feces into a tared 1.5 ml microcentrifuge tube and determine the actual weight.
2. Add nine weight volumes of TBE buffer (150 mM NaCl, 10 mM Tris, 1 mM EDTA, pH 7.4) and seal the vial.
3. Vortex the tube(s) 3-4 times over a period of 30 minutes.
4. Centrifuge for 5 minutes using a microcentrifuge set at 14,000 rpm.
5. Save the supernatant (extract) for testing. Assume that this represents a 10-fold dilution of the feces.
6. Extracts may be frozen at or below -20°C and used after thawing.

When testing extracts, optimal dilutions should be determined empirically. To avoid matrix effects, however, the extracts should be diluted at least an additional 10-fold in diluent SB50-1 prior to assay (100-fold final dilution). Test 100 µl of the diluted extracts as described in the Procedure section.

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 standards and diluted samples into appropriate wells. We recommend that standards and samples be tested in duplicate.
3. Incubate on a plate shaker at 150 rpm and 25°C for 45 minutes.
4. Empty and wash the microtiter wells 5 times with 1x Wash Solution using a plate washer (400 µl/well).
5. Dispense 100 µl of diluted HRP conjugate into the wells.
6. Incubate on a plate shaker at 150 rpm and 25°C for 45-minutes.
7. Empty and wash the microtiter wells 5 times with 1x Wash Solution using a plate washer (400 µl/well).
8. If necessary, strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
9. Dispense 100 µl of TMB into each well.
10. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
11. After 20 minutes stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix. It is important to make sure that all the blue color changes to yellow.
13. Read absorbance at 450 nm with a plate reader within 5 minutes.

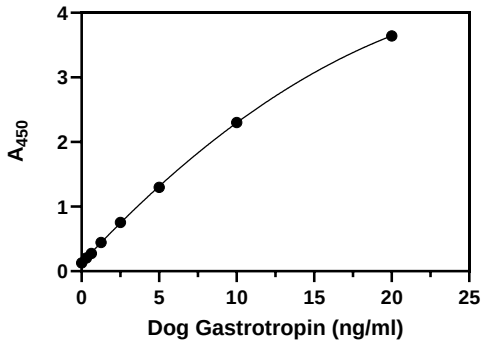
RESULTS

1. Using curve fitting software, graph the absorbance values of the standards on the Y-axis versus gastrotropin concentration on the X-axis.
2. Fit the curve to a second order polynomial (quadratic) equation and derive the concentration of gastrotropin in the diluted samples.
3. Multiply derived values by the final dilution factor(s) to determine gastrotropin concentration per gram of feces.
4. If the absorbance values of diluted samples fall outside the standard curve, samples should be further diluted and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve is shown below. This curve is for illustration only.

Gastrotropin (ng/ml)	A_{450}
20	3.643
10	2.303
5	1.298
2.5	0.756
1.25	0.446
0.625	0.275
0.313	0.204
0	0.130



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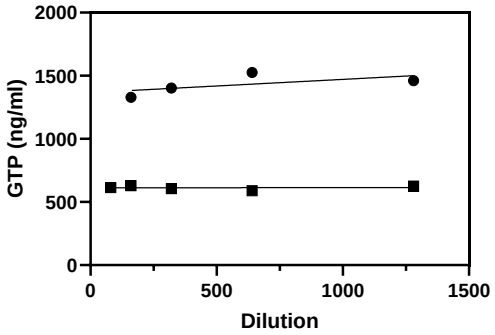
PERFORMANCE

Linearity: To assess the linearity of the assay, extracts with gastrotropin levels of 613 and 1430 ng/g were serially diluted with diluent SB50-1 to give values within range of the assay.



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For technical assistance please email us: techsupport@lifediagnostics.com

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