Canine Fatty Acid Binding Protein 2 Intestinal FABP2, ELISA Kit

Catalog #: BG-CAN10949 (96 wells)

User Manual

This kit is designed to quantitatively detect the levels of Canine FABP2 in serum/ plasma, cell lysates and other suitable sample solution.

Manufactured and Distributed by:

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Important notes

Before using this product, please read this manual carefully; after reading the subsequent contents of this manual, please note the following specially:

- The operation should be carried out in strict accordance with the provided instructions.
- Store the unused strips in a sealed foil bag at 2-8°C.
- Always avoid foaming when mixing or reconstituting protein solutions.
- Pipette reagents and samples into the center of each well, avoid bubbles.
- The samples should be transferred into the assay wells within 15 minutes of dilution.
- We recommend that all standards, testing samples are tested in duplicate.
- Using serial diluted sample is recommended for first test to get the best dilution factor.
- If the blue color develops too light after 15 minutes incubation with the substrate, it may be appropriate to extend the incubation time (Do not over-develop).
- Avoid cross-contamination by changing tips, using separate reservoirs for each reagent.
- Avoid using the suction head without extensive wash.
- Do not mix the reagents from different batches.
- Stop Solution should be added in the same order of the Substrate Solution.
- TMB developing agent is light-sensitive. Avoid prolonged exposure to the light.

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Intended use

The kit is used to quantify the Canine FABP2 in cell culture supernatant and other suitable sample solution.

Standard range	62.5 - 2000 pg/ml
Assay time	90 min
Validity	Six months
Store at	2-8 °C

Assay principle

The Canine FABP2 ELISA Kit is based on standard sandwich enzyme-linked immunosorbent assay technology. Anti-Canine FABP2 specific antibody has been pre-coated onto 96-well plate. Canine FABP2 present in the standards/ samples bind to the capture antibody. Subsequently, HRP conjugated anti-Canine FABP2 detection antibody is added to form an Ab-Ag-Ab sandwich. After a washing step, HRP substrate, TMB is added. HRP (horse radish peroxidase) converts TMB to a blue colored product which changes to yellow after the addition of acidic stop solution. The density of yellow color is directly proportional to the amount of Canine FABP2 captured on plate.

Materials supplied

1. Canine FABP2 standard*:	6 vials
2. 96-well plate pre-coated with anti-Canine FABP2 Ab:	1
3. Sample Diluent:	6 ml
4. HRP conjugated reagent:	10 ml
6. Chromogen Solution A:	6 ml
7. Chromogen Solution B:	6 ml
8. Stop Solution:	6 ml
9. 20 × Wash Buffer:	25 ml
10. Plate sealer	2
11. Package insert	1

Note: **6 ready to use standard vials with 62.5, 125, 250, 500, 1000, 2000 pg/ml of Canine FABP2.*

Materials required but not supplied

- 1x PBS.
- Standard plate reader capable of measuring absorbance at 450 nm.
- Adjustable pipettes and disposable pipette tips.
- Multi-channel pipettes, manifold dispenser or automated microplate washer.
- Distilled water.
- Absorbent paper.
- Materials used for sample preparation.

Sample Preparation and storage

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

- Cell culture supernatant, tissue lysate or body fluids: Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C
- Serum: Allow the serum to clot in a serum separator tube (about 4hours) at room temperature. Centrifuge at approximately 2000 X g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20°C.
- Plasma: Collect plasma using heparin as an anticoagulant. Centrifuge for 15 min at 2000 x g within 30 minutes of collection. Analyze immediately or aliquot and store frozen at -20°C. EDTA and citrate are not recommended as the anticoagulant.

Reagent Preparation

Important: Bring all kit components and samples to room temperature (18-25 °C) before use.

Wash Buffer

- If crystals have formed in the 20X wash buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
- Dilute 25 ml Wash Buffer Concentrate (20X) to a total volume of 500 ml with distilled water.

Assay Procedure

Canine FABP2 standard curve should be run for each experiment. The user will decide sample dilution factor by rough estimation of Canine FABP2 concentration in samples.

1. Add 50 µl of sample or standards per well. Add 50 µl of the sample diluent into the control well (Zero well).
   Note: *We recommend that each Canine FABP2 standard solution and each sample is measured in duplicate.*
2. Add 100 µl of HRP Conjugate to each well. Mix well. **Mixing well in this step is important**. Cover and incubate the plate at 37°C for 1 hour.
3. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or auto-washer. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
4. Add 50 µl of chromogen solution A and 50 µl of chromogen solution B in to each well. Mix well. Alternatively, pre-mix equal volumes of chromogen solution A and chromogen solution B and add 100 µl to each well. Cover and incubate at 37°C for 15 min (or until a gradient develops and you see visible color in the 2nd lowest standard concentration well). Protect from light. Do not over-develop.
5. Add 50 µl Stop Solution to each well. Mix well.
6. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader immediately.

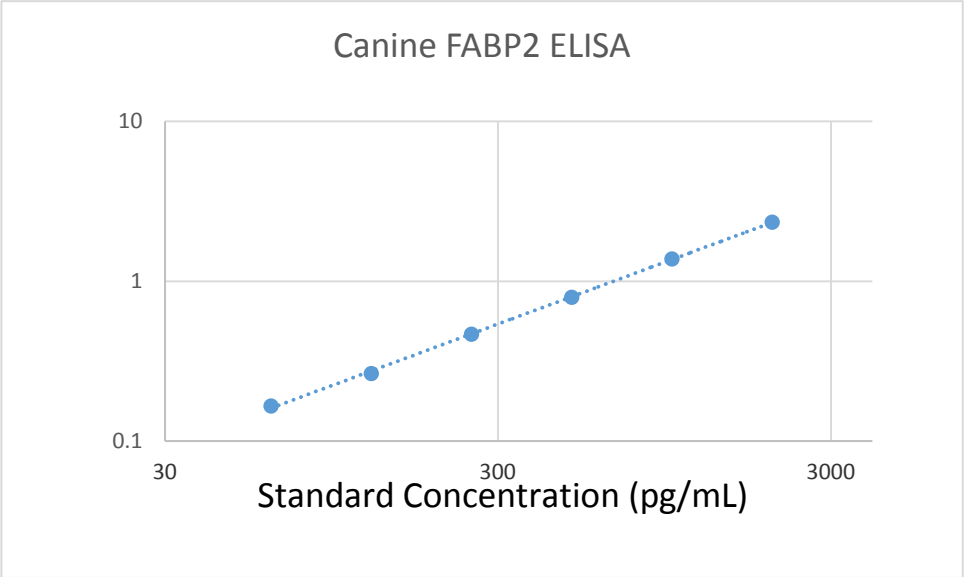
Result calculation

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Canine FABP2 concentration of the samples can be interpolated from the standard curve.

Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution

Typical data

This standard curve was generated at the Novatein Biosciences laboratory for demonstration purpose only. A standard curve must be run with each assay.



Sensitivity

The sensitivity or minimum detectable dose (MDD) of Canine FABP2 was determined to be 3.5 pg/ml. MDD is defined as the Canine FABP2 concentration resulting in an $O.D._{450}$ value that is 2 standard deviations higher than blank.

Spiking and Recovery

Recovery was determined by spiking the following matrices with various concentrations of canine FABP2.

Sample Type	Average Recovery (%)	Range (%)
Cell lysate	94.6	90-102
Serum	96.5	92-101

Reproducibility

- Inter-assay- <8.7%

- Intra-assay- <4.6%

Specificity

This kit recognizes both natural and recombinant Canine FABP2

Sample Dilution

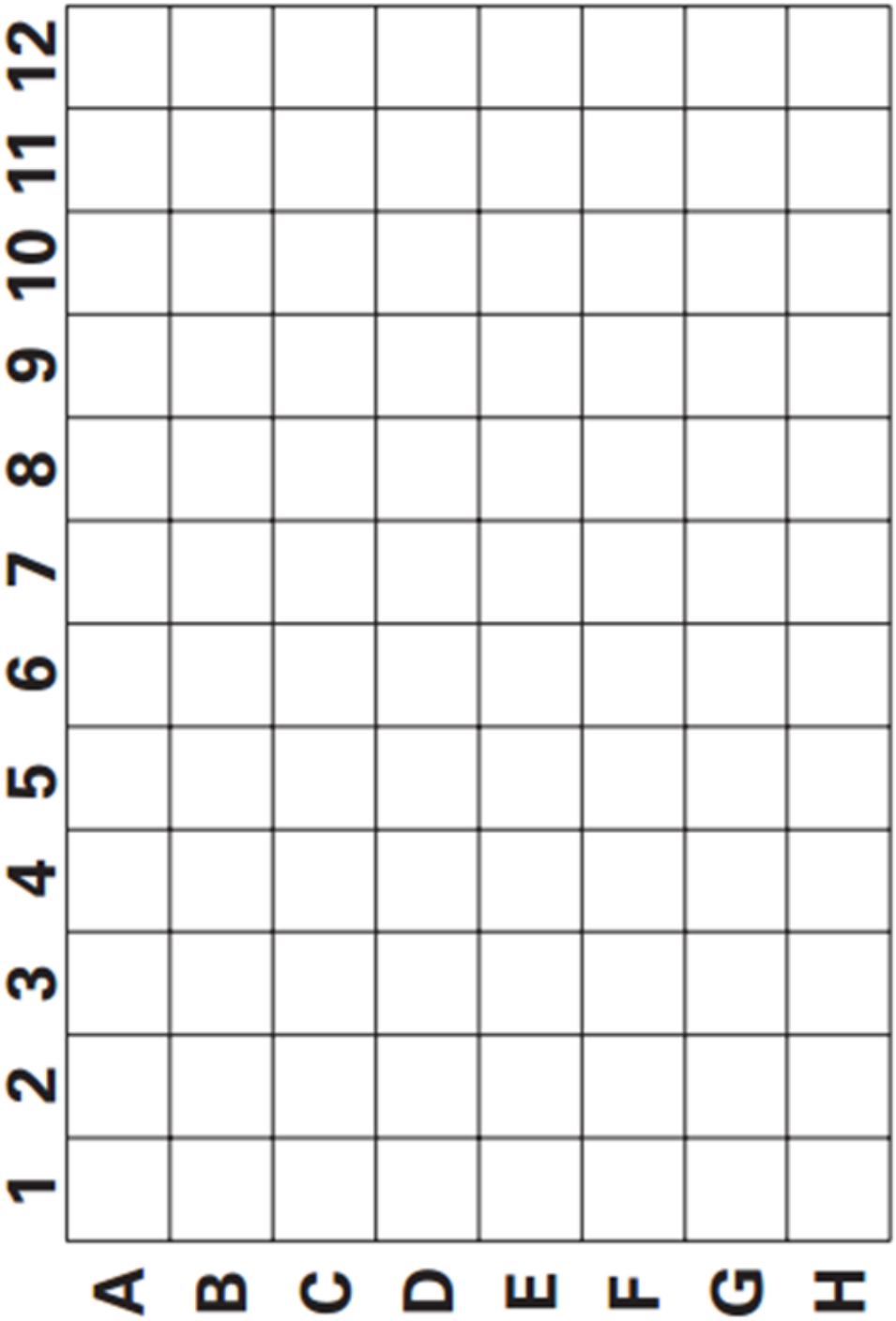
Levels of Canine FABP2 may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator. If required, samples should be diluted in the sample diluent.

For trouble shooting information please visit the following website:
http://www.novateinbio.com/en/content/15-tech-info OR

email us at techsupport@novateinbio.com

Notes

Plate Layout



Troubleshooting Information

High Background

Probable Cause:	Solution/ Action
High incubation temperature:	Incubate at room temperature (25 °C) throughout the procedure
Insufficient washing of the plate:	Fill the wells with wash buffer and aspirate completely for the next wash Increase the number of washes Add soak time (20-30 seconds) in between the washes Use automated plate washer, if available and check that all the channels are operating properly
Concentrated streptavidin-HRP	Streptavidin-HRP was not diluted properly Dilute the streptavidin-HRP as mentioned in the manual
Light exposure during substrate incubation	The TMB substrate is light sensitive and turns to blue color in the presence of light. The incubation must be carried out in dark.
Stop solution not added	Color will continue to develop if stop solution is not added
Diluents came with the kit were not used	Standards/ sample, detection antibody and streptavidin-HRP must be diluted in the respective buffers came with the kit. Do not use buffers from other kits
Contaminated solutions	Prepare fresh working solutions

Poor Standard Curve

Probable Cause:	Solution/ Action
Improper standard reconstitution:	Spin the vial briefly before opening Reconstitute the standard as mentioned in the manual. After reconstitution, leave it atleast for 10 minutes at room temperature Do not store and reuse diluted standards
Curve fitting problem:	Log transform the values on both axes Use 4-PL/ 5-PL curve fitting programs
Incubation temperature/ time	Use the recommended standard incubation conditions
Poor dilutions	Pipetting error. Check pipetting technique and calculations. Use calibrated pipettes.

No Signal

Probable Cause:	Solution/ Action
Omission of reagent(s):	Read the manual entirely. Check that all the reagents are added in the correct order as stated in the manual
Incorrect detection antibody was used:	Use the detection antibody came with the kit
Chromogen solutions were mixed improperly	Use the recommended procedure to prepare the TMB substrate
HRP inhibitor in sample/ buffers	Check that the samples/ buffers do not have sodium azide as it will inhibit peroxidase reaction.
Vigorous washing	If the washing is done manually, pipette the wash buffer gently.
Dried wells	Do not allow the wells to dry out during the assay. Seal with the supplied adhesive cover during incubations
Improper plate reader settings	Check the wavelength and read the plate again

Erratic duplicate OD values

Probable Cause:	Solution/ Action
Insufficient washing of the plate	Fill the wells with wash buffer and aspirate completely for the next wash Increase number of washes Add soak time (20-30 seconds) in between the washes Use automated plate washer, is available and check that all the channels are functioning properly
Poor dilutions	Pipetting error. Check pipetting technique and calculations. Use calibrated pipettes.
Improper mixing of samples/ buffers	Mix the samples well before pipetting Thoroughly mix the working solutions of detection antibody/ streptavidin-HRP
Contamination from other wells	Do not reuse the adhesive covers from previous assay setups Change pipette tips during reagent addition. If same pipette tip is being used to dispense reagents, care should be taken, not to touch the solution in the well
Precipitates in the samples/ buffer	If precipitates are visible in wash buffer concentrate, keep it at 37 ^{o}C for 10-15 minutes until no precipitates are visible Centrifuge the samples to remove particulate matter
Dried wells	Do not allow the wells to dry out during the assay. Seal with the supplied adhesive cover during incubations