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Datasheet for ABIN956139

FABP3 ELISA Kit

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Overview

Quantity:	96 tests
Target:	FABP3
Reactivity:	Dog
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	The Dog H-FABP ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA).
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Characteristics:	The assay uses affinity purified anti-dog H-FABP antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-dog H-FABP antibodies for detection.
Components:	Anti-dog H-FABP antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8) Enzyme Conjugate Reagent, 11 mL Calibrator (lyophilized), containing 60 ng/mL dog H-FABP 10X Diluent (25 mL) 20X Wash Solution (50 mL) TMB Reagent (One-Step), 11 mL Stop Solution (1N HCl), 11 mL.

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Product Details

Material not included:	Precision pipettes and tips Distilled or de-ionized water Polypropylene or glass tubes Vortex mixer Absorbent paper or paper towels Micro-Plate incubator/shaker mixing speed of ~150 rpm A microtiter plate reader capable of measuring absorbance at 450 nm, with a bandwidth of 10 nm or less and an OD range of 0-4 OD Graph paper (PC graphing software is optional)

Target Details

Target:	FABP3
Alternative Name:	H-FABP (FABP3 Products)
Pathways:	Monocarboxylic Acid Catabolic Process

Application Details

Plate:	Pre-coated
Protocol:	The test sample is diluted and incubated in the microtiter wells for 45 minutes. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 30 minutes. This results in H-FABP molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies and TMB Reagent is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. The concentration of H-FABP is proportional to the optical density of the test sample.
Sample Preparation:	General Note: In plasma samples from a dog ischemia-reperfusion model we found that peak H-FABP levels of ~85 ng/mL were achieved 2h after reperfusion. Baseline levels were approximately 1 ng/mL. We suggest that samples initially be tested after a 5-fold dilution in 1x sample diluent. 1. Dispense 240 µL of 1x diluent into separate tubes. 2. Pipette and mix 60 µL of each serum/plasma sample into a tube containing 240 µL of diluent. This provides a 5 fold diluted sample.
Assay Procedure:	1. Secure the desired number of coated wells in the holder.

	2. Dispense 100 µL of calibrators and samples into the wells (we recommend that samples be tested in duplicate). 3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes. 4. Remove the incubation mixture by flicking plate contents into an appropriate Bio-waste container. 5. Wash and empty the microtiter wells 5 times with 1X wash solution. This may be performed using either a plate washer (350 µL/well) or with a squirt bottle. The entire wash procedure should be performed as quickly as possible. 6. Strike the wells sharply onto adsorbent paper or paper towels to remove all residual droplets. 7. Add 100 µL of enzyme conjugate reagent into each well. 8. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 30 minutes. 9. Wash as detailed in 4 to 5 above. 10. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets. 11. Dispense 100 µL of TMB Reagent into each well. 12. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 20 minutes. 13. Stop the reaction by adding 100 µL of Stop Solution to each well. 14. Gently mix. It is important to make sure that all the blue color changes to yellow. 15. Read the optical density at 450 nm with a microtiter plate reader within 15 minutes.
Calculation of Results:	1. Calculate the average absorbance values (A450) for each set of reference calibrators, and samples. 2. Construct a calibration curve by plotting the mean absorbance obtained from each reference calibrator against its concentration in ng/mL on linear graph paper, with absorbance values on the vertical or Y-axis and concentration on the horizontal or X-axis. 3. Using the mean absorbance value for each sample, determine the corresponding concentration of H-FABP in ng/mL from the calibration curve. 4. Multiply the derived concentration by the dilution factor to determine the actual concentration of H-FABP in the serum/plasma sample. 5. If available, PC graphing software may be used for the above steps. 6. If the OD450 values of samples fall outside of the calibration curve, samples should be diluted appropriately and re-tested.
Restrictions:	For Research Use only

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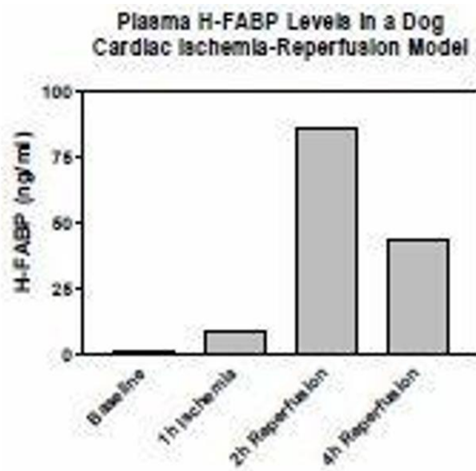
Handling

Storage:	4 °C
Storage Comment:	The unused kit should be stored at 4°C and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable until the expiration date provided that the components are stored as described above.
Expiry Date:	The expiry date is stated on the label.

Images



Western Blotting

Image 1.

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