Datasheet for ABIN7041578

Hemoglobin ELISA Kit

1 Image

Go to Product page

Overview

Quantity:	96 tests
Target:	Hemoglobin
Reactivity:	Dog
Method Type:	Sandwich ELISA
Detection Range:	12.5 ng/mL - 400 ng/mL
Minimum Detection Limit:	12.5 ng/mL
Application:	ELISA

Product Details

Purpose:	The kit is a sandwich enzyme immunoassay for the in vitro quantitative measurement of hemoglobin in dog serum, plasma.
Sample Type:	Plasma, Serum, Urine
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	• ELISA Micro Plate antibody coated • Enzyme Conjugated Detection Antibody (100X) • Calibrator • Diluent Concentrate (5X) • Wash Solution Concentrate (20X) • Chromogen - Substrate Solution • STOP Solution

Product Details

Material not included:	1. Microplate reader with 450 ± 10nm filter. 2. Precision single or multi-channel pipettes and disposable tips. 3. Microcentrifuge tubes for diluting samples. 4. Squirt bottle or Microtitre washer 5. Deionized or distilled water. 6. Container for Wash Solution 7. Centrifuge for sample collection 8. Anticoagulant for plasma collection 9. Incubator capable of maintaining 37 °C. 10. Microplate shaker

Target Details

Target:	Hemoglobin
Abstract:	Hemoglobin Products

Application Details

Sample Volume:	100 µL
Assay Time:	1 - 2 h
Plate:	Pre-coated
Protocol:	In this assay the hemoglobin (HM) present in samples reacts with the anti-HM antibodies, which have been adsorbed to the surface of polystyrene microtitre wells. After the removal of unbound proteins by washing, anti-HM antibodies conjugated with horseradish peroxidase (HRP) are added. These enzyme-labeled antibodies form complexes with the previously bound HM. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme varies directly with the concentration of HM in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of HM in the test sample. The quantity of HM in the test sample can be interpolated from the standard curve constructed from the standards, and corrected for sample dilution.
Reagent Preparation:	• Bring all reagents to room temperature (16°C to 25°C) before use. • Diluent Concentrate - The Diluent Solution supplied is a 5X Concentrate and must be diluted 1/5 with distilled or deionized water (1 part buffer concentrate, 4 parts dH2O). • Wash Solution Concentrate - The Wash Solution supplied is a 20X Concentrate and must be diluted 1/20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH2O). Crystal formation in the concentrate may occur when storage temperatures are low.

Warming of the concentrate to 30-35°Cbefore dilution can dissolve crystals.

- Enzyme-Antibody Conjugate - Calculate the required amount of working conjugate solution for each microtitre plate test strip by adding 10 µL Enzyme-Antibody Conjugate to 990 µL of 1X Diluent for each test strip to be used for testing. Dilute immediately before use and protect from light. Mix uniformly, but gently. Avoid foaming.
- Pre-coated ELISA Micro Plate - Ready to use as supplied. Unseal foil pouch and remove plate from pouch. Remove all strips and wells that will not be used in the assay and place back in pouch and re-seal along with desiccant.
- Calibrator – Prepare according to the lot specific Certificate of Analysis.

Sample Preparation:

- It is recommended to use fresh samples without long storage, otherwise protein degradation and denaturationmay occur in these samples, leading to false results. Samples should therefore be stored for a short periodat 2 - 8 °C or aliquoted at -20 °C (≤1 month) or -80 °C (≤ 3 months). Repeated freeze-thawcycles should be avoided. Prior to assay, the frozen samples should be slowly thawed and centrifuged toremove precipitates.
- If the sample type is not specified in the instructions, a preliminary test is necessary to determinecompatibility with the kit.
- If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibilityof causing a deviation due to the introduced chemical substance.The recommended dilution factor is for reference only.
- Please estimate the concentration of the samples before performing the test. If the values are not in therange of the standard curve, the optimal sample dilution for the particular experiment has to be determined.Samples should then be diluted with PBS (pH =7.0-7.2).

Note:

The assay requires that each test sample be diluted before use. All samples should be assayed in duplicate each time the assay is performed. The recommended dilutions are only suggestions. Dilutions should be based on the expected concentration of the unknown sample such that the diluted sample falls within the dynamic range of the standard curve. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

Serum samples – Recommended starting dilution is 1/10,000. To prepare a 1/10,000 dilution of a sample, transfer 5 µL of sample to 495 µL of 1X diluent. This gives you a 1/100 dilution. Next, dilute the 1/100 by transferring 5 µL into 495 µL of 1X diluent. This gives you a 1/10,000 dilution. Mix thoroughly each stage.

Plasma samples – Recommended starting dilution is 1/10,000. To prepare a 1/10,000 dilution of a sample, transfer 5 µL of sample to 495 µL of 1X diluent. This gives you a 1/100 dilution. Next, dilute the 1/100 by transferring 5 µL into 495 µL of 1X diluent. This gives you a 1/10,000 dilution. Mix thoroughly each stage.

Assay Procedure:

1. All samples and standards should be assayed in duplicates.

Application Details

2. The Standards and the test sample(s) should be loaded into the ELISA wells as quickly as possible to avoid a shift in OD readings. Using a multichannel pipette would reduce this occurrence. Pipette 100 µL of Standard 0 (0.0 ng/mL) in duplicate Standard 1 (6.25 ng/mL) in duplicate Standard 2 (12.50 ng/mL) in duplicate Standard 3 (25 ng/mL) in duplicate Standard 4 (50 ng/mL) in duplicate Standard 5 (100 ng/mL) in duplicate Standard 6 (200 ng/mL) in duplicate

3. Pipette 100 µL of sample (in duplicate) into pre designated wells.

4. Incubate the micro titer plate at room temperature for thirty (30 ± 2) minutes. Keep plate covered and level during incubation.

5. Following incubation, aspirate the contents of the wells.

6. Completely fill each well with appropriately diluted Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with wash buffer, invert the plate then pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.

7. Pipette 100 µL of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at room temperature for thirty (30 ± 2) minutes. Keep plate covered in the dark and level during incubation.

8. Wash and blot the wells as described in Steps 5/6.

9. Pipette 100 µL of TMB Substrate Solution into each well.

10. Incubate in the dark at room temperature for precisely ten (10) minutes.

11. After ten minutes, add 100 µL of Stop Solution to each well.

12. Determine the absorbance (450 nm) of the contents of each well within 30 minutes. Calibrate the plate reader to manufacturer's specifications.

Restrictions:	For Research Use only

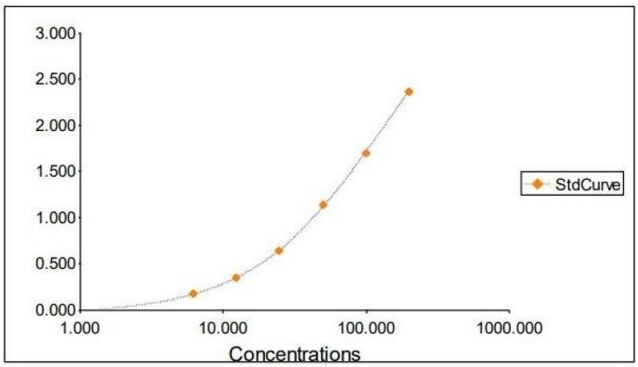
Handling

Storage:	4 °C
Storage Comment:	1. For unopened kit: All reagents should be stored according to the labels on the vials. All reagents should be stored at 4 °C for long term storage. Keep away from heat or direct sunlight. 2. For opened kits: the remaining reagents must be stored according to the above storage conditions. In addition, please return the unused wells to the foil pouch containing the desiccant and seal the foil pouch with the zipper. .

Handling

Expiry Date:	6 months

Images



ELISA

Image 1. Standard Curve Graph

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