Datasheet for ABIN6956459

Hemoglobin ELISA Kit

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Overview

Quantity:	96 tests
Target:	Hemoglobin
Reactivity:	Cow
Method Type:	Competition ELISA
Detection Range:	12.35 µg/mL - 1000 µg/mL
Minimum Detection Limit:	12.35 µg/mL
Application:	ELISA

Product Details

Purpose:	The kit is a competitive inhibition enzyme immunoassay technique for the in vitro quantitative measurement of hemoglobin in bovine serum, plasma, erythrocyte lysates.
Sample Type:	Cell Lysate, Plasma, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay has high sensitivity and excellent specificity for detection of Hemoglobin (HB)
Sensitivity:	5.57 µg/mL
Components:	• Pre-coated, ready to use 96-well strip plate, flat buttom • Plate sealer for 96 wells • Reference Standard • Standard Diluent • Detection Reagent A

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Product Details

- Detection Reagent B
- Assay Diluent A
- Assay Diluent B
- Reagent Diluent (if Detection Reagent is lyophilized)
- TMB Substrate
- Stop Solution
- Wash Buffer (30 x concentrate)
- Instruction manual

Target Details

Target:	Hemoglobin
Alternative Name:	Hemoglobin (HB) (Hemoglobin Products)

Application Details

Comment:	Information on standard material: The standard might be recombinant protein or natural protein, that will depend on the specific kit. Moreover, the expression system is E.coli or yeast or mammal cell. There is 0.05% proclin 300 in the standard as preservative. Information on reagents: The stop solution used in the kit is sulfuric acid with concentration of 1 mol/L. And the wash solution is TBS. The standard diluent contains 0.02 % sodium azide, assay diluent A and assay diluent B contain 0.01% sodium azide. Some kits can contain is BSA in them. Information on antibodies: The provided antibodies and their host vary in different kits.
Sample Volume:	50 µL
Assay Time:	2 h
Plate:	Pre-coated
Protocol:	1. Prepare all reagents, samples and standards, 2. Add 50µL standard or sample to each well. Then add 50µL prepared Detection Reagent A immediately. Shake and mix. Incubate 1 hour at 37 °C, 3. Aspirate and wash 3 times, 4. Add 100µL prepared Detection Reagent B. Incubate 30 minutes at 37 °C,

5. Aspirate and wash 5 times,
6. Add 90μL Substrate Solution. Incubate 10-20 minutes at 37 °C,
7. Add 50μL Stop Solution. Read at 450 nm immediately.

Reagent Preparation:

1. Bring all kit components and samples to room temperature (18-25 °C) before use.
2. Standard - Reconstitute the Standard with 0.5 mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently(not to foam). The concentration of the standard in the stock solution is 1,000 μg/mL. Please prepare 5 tubes containing 0.6 mL Standard Diluent and produce a triple dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Set up 5 points of diluted standard such as 1,000 μg/mL, 333.33 μg/mL, 111.11 μg/mL, 37.04 μg/mL, 12.35 μg/mL, and the last EP tubes with Standard Diluent is the blank as 0 μg/mL.
3. Detection Reagent A and Detection Reagent B - Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute to the working concentration with Assay Diluent A and B, respectively (1:100).
4. Wash Solution - Dilute 20 mL of Wash Solution concentrate (30x) with 580 mL of deionized or distilled water to prepare 600 mL of Wash Solution (1x).
5. TMB substrate - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Note:

1. Making serial dilution in the wells directly is not permitted.
2. Prepare standard within 15 minutes before assay. Please do not dissolve the reagents at 37 °C directly.
3. Detection Reagent A and B are sticky solutions, therefore, slowly pipette them to reduce the volume errors.
4. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals are completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10μL for once pipetting.
5. The reconstituted Standards, Detection Reagent A and Detection Reagent B can be used only once.
6. If crystals have formed in the Wash Solution concentrate (30x), warm to room temperature and mix gently until the crystals are completely dissolved.
7. Contaminated water or container for reagent preparation will influence the detection result.

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

Application Details

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).

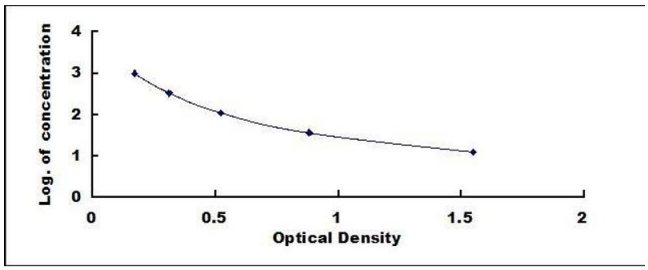
Assay Precision:	Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level of target were tested 20 times on one plate, respectively. Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level of target were tested on 3 different plates, 8 replicates in each plate. CV(%) = SD/meanX100 Intra-Assay: CV < 10% Inter-Assay: CV < 12%
Restrictions:	For Research Use only

Handling

Precaution of Use:	The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.
Storage:	4 °C/-20 °C
Storage Comment:	1. For unopened kit: All reagents should be stored according to the labels on the vials. The Standard, Detection Reagent A, Detection Reagent B, and 96-well Strip Plate should be stored at -20 °C upon receipt, while the other reagents should be stored at 4 °C. 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. .
Expiry Date:	6 months

Publications

Product cited in:	López, Villar, Queirós, Vicente, Mateos-Hernández, Díez-Delgado, Contreras, Alves, Alberdi, Gortázar, de la Fuente: "Comparative Proteomics Identifies Host Immune System Proteins Affected by Infection with Mycobacterium bovis." in: **PLoS neglected tropical diseases**, Vol. 10, Issue 3, pp. e0004541, (2016) (PubMed).



ELISA

Image 1. Typical standard curve

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