Mouse Hemoglobin ELISA Kit

Mouse Hemoglobin ELISA Kit is an Enzyme Immunoassay kit for the quantification of Mouse Hemoglobin in serum and plasma.

Catalog number: ARG82321

Package: 96 wells

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INTRODUCTION

Hemoglobin or haemoglobin (spelling differences), abbreviated Hb or Hgb, is the iron-containing oxygen-transport metalloprotein in the red blood cells (erythrocytes) of almost all vertebrate (the exception being the fish family Channichthyidae) as well as the tissues of some invertebrates. Hemoglobin in blood carries oxygen from the lungs or gills to the rest of the body (E.g., the tissues). There it releases the oxygen to permit aerobic respiration to provide energy to power the functions of the organism in the process called metabolism. A healthy individual has 12 to 20 grams of hemoglobin in every 100 mL of blood. [Provide by Wikipedia: MMP9]

PRINCIPLE OF THE ASSAY

This Mouse Hemoglobin Assay Kit is a simple assay that measures the amount of mouse hemoglobin in the samples. The hemoglobin present in the sample binds with anti-hemoglobin antibodies adsorbed to the surface of the microplate. In the following step, another specific anti-hemoglobin antibody binds, in turn, to the immobilized hemoglobin. The second antibody is conjugated with horseradish peroxidase (HRP). In the closing substrate reaction, the hemoglobin levels of the samples can be measured by color intensity.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all reagent at 2-8°C upon receiving. Do not freeze or hold it at temperature above 25°C. Do not use kit components past kit expiration date.

Component	Quantity	Storage information
Antibody Coated Microplate	8 x 12 strips	4°C
5X Diluent Buffer	50 mL	4°C
100X Enzyme Conjugate	150 µL	4°C
TMB Substrate	12 mL	4°C
Stop Solution	12 mL	4°C
20X Wash Buffer	50 mL	4°C
Standard (mouse hemoglobin, 122.4 µg/mL)	1 vial	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 450 / 630 nm
- Centrifuge and centrifuge tube
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature.
- Avoid air bubbles in the wells as this could result in lower binding efficiency and higher CV% of duplicate reading.
- Briefly spin down the all vials before use.
- If crystals are observed in the 20X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.
- Include a standard curve each time the assay is performed.
- Run both standards and samples in at least duplicates (triplicate is recommended).

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2000 x g for 10 minutes at 4°C. Store frozen at -20°C or lower. Avoid freeze-thaw cycles.

Plasma: Collect blood with EDTA, heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C. Store frozen at -20°C or lower. Avoid freeze-thaw cycles.

Note:

- Azide and thimerosal at concentrations higher than 0.1% inhibit the enzyme reaction.
- Serum and plasma sample need to be diluted with 1X Diluent Buffer. A sample dilution of 1:4000 is generally suitable. To prepare the 1:4000 dilution, first prepare a 1:40 dilution by mixing 5 µL of sample with 195 µL of 1X Diluent Buffer. Next, mix 5 µL of the 1:40 dilution with 495 µL of 1X Diluent Buffer to achieve the 1:4000 dilution.
- Since hemoglobin levels can vary, dilution ratios may need to be adjusted as appropriate to ensure that the results fall within the range of the standard curve.

REAGENT PREPARATION

- 1X Diluent Buffer: Dilute 5X Diluent buffer into distilled water to yield 1X Diluent buffer (E.g., add 20 mL of 5X Diluent Buffer into 80 mL of distilled water to a final volume of 100 mL). 1X Diluent Buffer is stable for at least

1 week at 2-8°C.

- **1X Wash Buffer:** Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer (E.g., add 25 mL of 20X Wash Buffer into 475 mL of distilled water to a final volume of 500 mL). 1X Wash Buffer is stable for at least 1 week at room temperature or at 2-8°C.
- **1X Enzyme Conjugate:** The 100X Enzyme Conjugate has to be diluted 1:100 with 1X Diluent Buffer. (E.g., add 10 µL of 100X Enzyme Conjugate into 990 µL of 1X Diluent Buffer to a final volume of 1 mL).
- **Standard:** Mix 2 µL of the Standard (122.4 µg/mL) with 610 µL of 1X Diluent Buffer to the conc. of 400 ng/mL. Diluted the standard as follow.

Standard tube	Hemoglobin (ng/mL)	1X Diluent Buffer (µL)	Standard stock, 400 ng/mL (µL)
S1	400	0	600
S2	200	300	300 of S1
S3	100	300	300 of S2
S4	50	300	300 of S3
S5	25	300	300 of S4
S6	12.5	300	300 of S5
S0	0	600	0

Note: Working standard should be prepared immediately prior to use.

ASSAY PROCEDURE

Prior to running the assay, all reagents should be brought to room temperature for at least 30 minutes. It is recommended that all samples and standards be assayed in duplicate.

1. Add **100 µL** of **diluted samples** or **each diluted Standard** into respective wells.
2. Cover the plate and incubate for **60 minutes** at **room temperature**.
3. Aspirate each well and wash, repeating the process 3 times for a total 4 washes. Wash by filling each well with **1× Wash Buffer (300 µL)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µL** of **1X Enzyme Conjugate** to each well. Mix well by repeated pipetting.
5. Cover the plate and incubate for **30 minutes** at **room temperature** in the dark.
6. Aspirate and wash plate as in step 3.
7. Add **100 µL** of **TMB Substrate** in each well.
8. Incubate for **10 minutes** at **room temperature** in the dark.
9. Add **100 µL** of **Stop Solution** to each well to stop the reaction.
10. Read the absorbance with a plate reader at **O.D. 450 nm.** (**O.D. 630 nm as reference**)
11. Measure $O.D._{450nm}$ value and subtract $O.D._{630nm}$ value.

CALCULATION OF RESULTS

1. Subtract zero point (S0) from all standards and unknowns to determine corrected absorbance.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
4. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.
6. Samples with high mouse hemoglobin concentrations (E.g., fall above the range of the assay) should be further diluted and rerun.

QUALITY ASSURANCE

Sensitivity

3.11 ng/mL