(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Hemoglobin (Hb) Assay Kit

Catalog No.: BC00044

Size: 100T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Hemoglobin (Hb) Assay Kit
Detection Method	Colorimetric
Sample Type	Tissue, Serum, Plasma, Whole blood, Urine,etc
Assay Type	Quantitative
Detection Instrument	Microplate reader(405-415nm,optimal detection wavelength 410nm)

Product Introduction

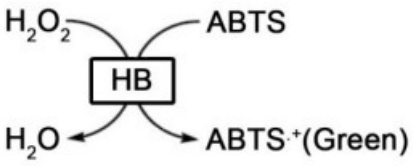
Hemoglobin (HB or HGB) is an iron-containing metalloprotein responsible for carrying oxygen in higher organisms. Hemoglobin is mainly found in red blood cells, accounting for 97% of the dry weight of red blood cells. In addition, hemoglobin also exists in other tissues as an antioxidant. It is precisely because of the presence of iron-containing hemoglobin that human blood is red. Hemoglobin in the human body is composed of four subunits, two α subunits and two β subunits, and each subunit is composed of a peptide chain and a heme molecule. Under physiological conditions, the peptide chain will coil and fold into a sphere, wrapping the heme molecule inside. The spherical structure formed by this peptide chain is also called globin. Heme molecule is a small molecule with a porphyrin structure. In the center of the porphyrin molecule, the nitrogen atoms on the four pyrrole rings in the porphyrin are coordinated with a ferrous ion. When hemoglobin is not bound to an oxygen molecule, a water molecule is coordinated with the ferrous ion from the bottom of the porphyrin ring; and when hemoglobin carries oxygen, the oxygen molecule replaces the water molecule. This characteristic of hemoglobin enables red blood cells to transport oxygen. In lung tissue, hemoglobin can fully bind to oxygen and transport oxygen to peripheral tissues through red blood cells to maintain cell vitality. In addition to the function of transporting oxygen molecules, hemoglobin also plays an important role in maintaining the normal morphology of red blood cells.
Hemoglobin is one of the most commonly used indicators for anemia screening and clinical diagnosis. A decrease in hemoglobin concentration is common in various types of anemia caused by bleeding, lack of iron, vitamin B12 or folic acid; while an increase in hemoglobin concentration is more common in polycythemia vera, cyanotic congenital heart disease, and diseases caused by various clinical causes such as heat stroke and dehydration. Therefore, hemoglobin content is an important clinical indicator for the diagnosis of various diseases such as anemia, polycythemia and dehydration.

Product Features

● This kit has the advantages of convenient operation, high sensitivity, low cost, wide linear range, and suitable for high-throughput detection. The lower limit of detection can reach below 0.1 mg/dl, and the upper limit of detection can reach 80 mg/dl.

Principle

Hemoglobin (HB) catalyzes hydrogen peroxide to produce oxygen and oxidizes the colorimetric reagent to produce a green product with a maximum absorption wavelength at 410nm. Refer to the figure below :



Components

Serial number	Components	Size(100T)	Storage
Reagent 1	Chromogen	22ml	Store at -20℃, away from light.
Reagent 2	Hydrogen Peroxide Solution (30 ×)	12ml	Store at -20℃, away from light.
Reagent 3	Hemoglobin (100 mg/dl)	0.4ml	-20℃
Consumable 1	Microplate(96 wells)	1 plate	RT
Consumable 2	Plate Sealer	2 pieces	RT

Storage

The unopened kit can be stored at -20℃ for 12 months.

Experimental Preparation

- Sample processing

1. Preparation of plasma, serum and whole blood samples

a. Plasma samples: Collect whole blood, add anticoagulant heparin or sodium citrate, centrifuge at 700-1000 x g at 4°C for 10 minutes, and aspirate the top layer of yellow plasma. Plasma samples can be stored at -80°C for one month to avoid repeated freezing and thawing.

b. Serum samples: Collect whole blood, let the blood coagulate automatically at room temperature for 30 minutes, centrifuge at 2000 x g for 5 minutes, and aspirate the yellow serum on the top layer. It can be stored at -80ºC for one month. Serum and plasma samples do not need to be diluted before testing.

c. Whole blood samples: Collect whole blood, add anticoagulant heparin or sodium citrate, and store at 4°C for testing. Whole blood samples need to be appropriately diluted for testing.

2. Preparation of tissue samples: Weigh an appropriate amount of fresh or frozen tissue. Wash several times with PBS (pH7.4) containing 2% EDTA or 0.16mg/ml heparin to remove excess red blood cells and blood clots. Use a glass homogenizer or other appropriate homogenization equipment to fully homogenize the tissue according to the ratio of tissue weight (g) to homogenate solution (PBS pH7.4, containing 2% EDTA or 0.16mg/ml heparin) volume (ml) of 1:10. After homogenization, transfer to a centrifuge tube, centrifuge at 10,000 x g at 4ºC for 10 minutes, and take the supernatant as the sample to be tested.
3. Preparation of urine samples: For the detection of urine samples, it is generally suitable to detect urine samples with high hemoglobin content. Normal urine samples may not be suitable for this kit due to too low hemoglobin content. Urine can be placed for 10-15 minutes and then directly measured.

- Preparation of the assay kit

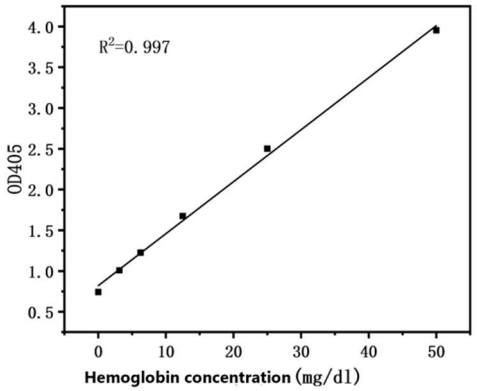
Prepare distilled water or saline to dilute the 100mg/dl hemoglobin standard provided by the kit to the required concentration of the standard curve. For the first test, you can set a hemoglobin standard curve of 0 , 3.125 , 6.25 , 12.5 , 25 , and 50mg/dl. After the hemoglobin concentration range of the sample is determined, you can select a hemoglobin standard curve with an appropriate concentration range according to the situation .

Operation process

1. Take 5μl of standard or sample (5-10μl) in a clean 1.5ml centrifuge tube. Note: If the sample to be tested is tissue homogenate, 10μl should be added for testing.
2. Add 200μl of colorimetric reagent and then 20μl of hydrogen peroxide solution (30X).
3. After thorough mixing, react at room temperature (25°C) for 30-90 minutes (if the hemoglobin concentration in the sample is relatively high, incubate for 30 minutes; if the hemoglobin concentration in the sample is low, the reaction time can be appropriately increased, but note that the reaction should not exceed 90 minutes), and centrifuge at 1500 x g for 5 minutes.
4. Pipette 200μl of supernatant from each tube into a clean 96-well plate. Be careful to avoid creating bubbles when transferring liquid. Alternatively, pipette 100-200μl from each tube into a 96-well plate as appropriate.
5. Measure the absorbance at 410nm (405-415nm can also be detected). To obtain ideal test results, the absorbance measurement should be completed within 20 minutes after the reaction is completed.

Result calculation

1. Calculate the hemoglobin concentration in the sample based on the standard curve. (The molecular weight of hemoglobin is about 64.5kd, and 1mg/dl is about 0.156μM). The following standard curve is for reference only:



2. If the absorbance value detected by the sample is higher than the upper limit of detection, the sample needs to be appropriately diluted before testing; if the hemoglobin concentration in the sample is too low, please increase the sample volume appropriately and extend the reaction time to 90 minutes. If the test data obtained is still not within the range of the standard curve, it means that the sample is not suitable for testing using this kit.

Notes

1. Substances that affect redox reactions, such as DTT and mercaptoethanol, should not be added to the sample, and detergents such as Tween, Triton and NP-40 should not be added .

2. **The reaction of this kit should be carried out at room temperature (about 25 ° C). Too high a temperature will have a greater impact on the test results .**

3. The anticoagulants (sodium EDTA, sodium citrate, sodium heparin) and bilirubin in the samples tested by this kit have no obvious effect on the test results, and the proteins and lipids in the plasma samples to be tested have little effect on the experimental results .

4. The color developing reagent is irritating to the human body, please take appropriate protective measures .

5. Hydrogen peroxide solution (30 ×) is corrosive. Please be careful when handling it and ensure effective protection to avoid direct contact with the human body. Be careful to avoid corrosion of other items .

6. This product is for Research Use Only, and shall not be used for clinical diagnosis or treatment, food or medicine, or stored in ordinary residences.