Hemoglobin Assay Kit

Catalog No.: abx298879

Size: 100 Assays

Storage: Store all components in the dark at 4°C.

Application: For quantitative detection of Hemoglobin concentrations in serum.

Detection Range: 1 µg/L – 100 µg/L

Introduction: Hemoglobin, also spelled haemoglobin and abbreviated Hb, is the iron-containing oxygen-transport metalloprotein in the red blood cells of the blood in vertebrates and other animals. In mammals the protein makes up about 97% of the red cell's dry content, and around 35% of the total content (including water). Hemoglobin transports oxygen from the lungs or gills to the rest of the body, such as to the muscles, where it releases its load of oxygen. Hemoglobin also has a variety of other gas-transport and effect-modulation duties, which vary from species to species, and may be quite diverse in invertebrates. The Hemoglobin can react with O-tolidine. The products can be measured at a colorimetric readout at 435 nm.

Abbexa's Hemoglobin Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Hemoglobin concentrations. The dye reagents react with Hemoglobin to create an absorption maximum at 435 nm. The intensity of the color is proportional to the concentration of Hemoglobin, which can then be calculated.

Kit components

1. 96 well microplate
2. Dye Reagent: 1 vial
3. Dye Reagent Diluent: 5 mL
4. Reaction Buffer: 10mL
5. Standard: 1 vial

Materials Required But Not Provided

1. Microplate reader (435 nm)
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Timer

For Reference Only

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Dye Reagent Solution**

  Add 5 mL of Dye Reagent Diluent into the Dye Reagent vial and mix thoroughly to prepare the Dye Reagent Solution. If any precipitates are observed, warm the vial using a water bath until the precipitates have dissolved.

- **Standard Solution**

  Add 1 mL of distilled water into the Standard vial and mix thoroughly. Ensure that the Standard has completely dissolved. Take 0.1mL of this solution and add 0.9 mL of distilled water to prepare the Standard Solution (concentration 100 µg/L). Unused Standard Solution can be stored at 4°C.

2. Sample

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 1000 × g for 20 mins. If precipitate appears, centrifuge again. Assay immediately or aliquot and store at -20°C or -80°C. Avoid multiple freeze-thaw cycles. Use serum samples directly for detection.

B. Assay Procedure

Warm all reagents to 37°C prior to use.

If the expected concentration is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured concentration within the detection range of the kit.

1. Label 8 tubes with 100 µg/L, 50 µg/L, 25 µg/L, 12.5 µg/L, 6.25 µg/L, 3.13 µg/L, 1.56 µg/L, 0.78 µg/L. Add 1.5 mL of 100 µg/L Standard Solution to the 1st tube. Aliquot 0.5 mL of distilled water into each tube, excluding the first. Transfer 0.5 mL from the 1st tube to the 2nd tube and mix thoroughly, and so on.
2. Set the sample, standard and blank wells on the 96 well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 100 µL of Reaction Buffer to all wells.
4. Tap the plate gently to mix. Allow to stand for 10 minutes.
5. Add 10 µL of sample to the sample wells.
6. Add 10 µL of prepared standards to the standard wells.
7. Add 10 µL of distilled water to the blank wells.
8. Add 50 µL of Dye Reagent Solution to all wells.
9. Tap the plate gently to mix. Allow to stand for 5 minutes. Read and record absorbance at 435 nm.

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C. Calculations

Hemoglobin concentration per ml of sample:

$$\textbf{Hemoglobin Concentration } (\mu g/mL) = C_{Standard} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} = 100 \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}}$$

where:

$C_{Standard}$ Concentration of highest standard (100 µg/mL)

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