Hemoglobin Colorimetric Assay Kit

(Catalog # BN00496; 200 assays; Store at 4°C)

I. Introduction:

Hemoglobin (Hb) is an iron containing globular metalloprotein found primarily in red blood cells. It carries oxygen from the lungs to the rest of the body as oxyhemoglobin and then returns back to the lungs as deoxyhemoglobin. In most vertebrates, hemoglobin is in tetrameric form comprising two α-globin chains and two β-globin chains ($\alpha_2\beta_2$). Each chain is associated with a non-protein heme group. Aside from oxygen transport, hemoglobin can bind and transport other molecules like nitric oxide and carbon monoxide. Hemoglobin also plays an important role in maintaining the shape of the red blood cells. Alterations in the structure and binding capacity of hemoglobin are associated with many blood disorders such as sickle-cell anemia and thalassemia. Assay Genie's Hemoglobin Colorimetric Assay kit provides a quick and easy method for monitoring hemoglobin levels in a wide variety of samples. In this assay, the detector selectively converts heme into a stable chemical complex that absorbs maximally at 575 nm. The intensity of the color is directly proportional to the total concentration of hemoglobin in the sample. The kit can detect as low as 0.02 g/dl hemoglobin.

II. Application:

- Estimation of Hemoglobin in various biological samples

III. Sample Type:

- Blood, serum, plasma, etc.

IV. Kit Contents:

Components	BN00496	Cap Code	Part Number
Hemoglobin Detector	50 ml	NM	BN00496-1
Hemoglobin Standard (equivalent to 1 g/dl)	1 ml	Blue	BN00496-2

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom.
- Multi-well spectrophotometer

VI. Storage Conditions and Reagent Preparation:

Store kit at 4°C, protected from light. Briefly spin small vials prior to opening. Read entire protocol before performing the assay.

- **Hemoglobin Detector:** Ready to use. Store at 4°C. Bring to room temperature (RT) before use.
- **Hemoglobin Standard:** Ready to use. Aliquot and store at 4°C. Do not freeze. Keep on ice during use.

VII. Hemoglobin Assay Protocol:

1. **Sample Preparation:** Add 20 µl of the diluted* test sample into desired well(s) in a 96-well plate. Adjust the volume to 20 µl/well with dH_2O.
   ***Notes:**
   a) Whole blood must be diluted with dH_2O prior to running the assay. The recommended dilution is 5-10 fold. Normal hemoglobin concentration in human blood ranges from 12-18 g/dl.
   b) Plasma or serum do not need to be diluted before measuring. Normal hemoglobin concentration in human plasma and serum is ~0.03 g/dl.
2. **Standard Curve Preparation:** Add 0, 10, 20, 30, 40, and 50 µl of 1 g/dl Hemoglobin Standard into a series of wells in a 96-well plate to generate 0, 50, 100, 150, 200, and 250 mg/dl of hemoglobin/well. Adjust the volume to 200 µl/well with Hemoglobin Detector. Mix well. Avoid bubbles while mixing.
3. **Reaction:** Add 180 µl of Hemoglobin Detector to all the Sample wells. Mix well and incubate at RT for 15 min. Avoid bubbles while mixing.
4. **Measurement:** Measure the absorbance at 575 nm in end point mode.
5. **Calculation:** Subtract 0 Standard reading from all readings. Plot the Hemoglobin Standard Curve. Apply sample's corrected OD to Standard Curve to get B mg/dl of Hemoglobin in the sample well.

Sample Hemoglobin Concentration (C) = B X D X 10* mg/dl

Where: **B** is amount of Hemoglobin in the sample well from Standard Curve (mg/dl)
D is sample dilution factor

*Accounts for sample dilution in the well.

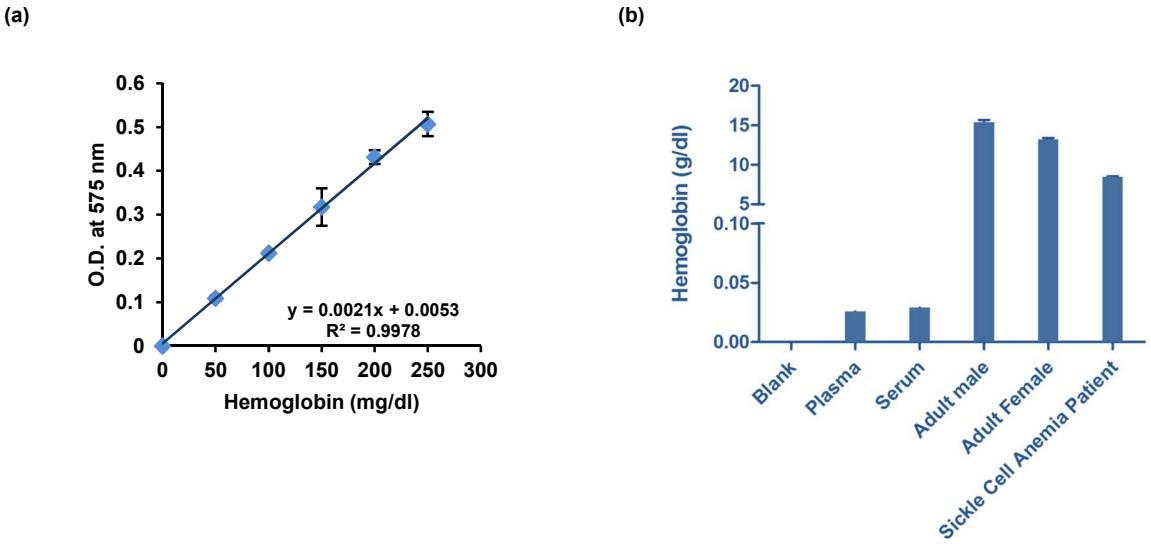

Figure: (a) Hemoglobin Standard Curve (0-250 mg/dl). **(b)** Estimation of hemoglobin concentration in human plasma, serum, adult male, adult female and sickle-cell anemia patient. Whole blood samples were diluted 10-fold. Assays were performed in triplicate following the kit protocol using 20 µl of the samples.

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