

Version 2e, Last updated 18 June 2025

ab234046

Hemoglobin Assay Kit

(Colorimetric)

For the measurement of hemoglobin in various biological samples.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Hemoglobin Assay Kit (Colorimetric) (ab234046) 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.

Prepare samples and standard curve.



Incubate samples with Hemoglobin Detector for 15 minutes at room temperature.



Measure the absorbance at 575 nm in end point mode.

2. Materials Supplied and Storage

Store kit at 4°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Hemoglobin Detector	50 mL	4°C	4°C
Hemoglobin Standard*	1 mL	4°C	4°C

*Do not freeze.

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

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

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:

www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Hemoglobin Detector

1. Ready to use as supplied.
2. Bring to room temperature before use.

5.2 Hemoglobin Standard

1. Ready to use as supplied.
2. Keep on ice during use.

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
 - Discard working standard dilutions after use as they do not store well.
1. Add 0, 10, 20, 30, 40 and 50 μL of 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.
 2. Adjust the volume to 200 μL /well with Hemoglobin Detector.
 3. Mix well. Avoid bubbles while mixing.

Standard#	Hemoglobin Standard (μL)	Hemoglobin Detector (μL)	Final volume standard in well (μL)
1	0	200	200
2	10	190	200
3	20	180	200
4	30	170	200
5	40	160	200
6	50	150	200

7. Sample Preparation

General sample information:

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

We recommend that you use fresh samples for the most reproducible assay.

1. Add 20 μL of the diluted test sample into desired well(s) in a 96-well plate.
2. For undiluted samples, adjust the volume to 20 μL /well with dH_2O .

Δ Note: 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.

Δ Note: Plasma or serum do not need to be diluted before measuring. Normal hemoglobin concentration in human plasma and serum is ~ 0.03 g/dL.

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

8.1 Reaction mix:

1. Add 180 μL of Hemoglobin Detector to all the Sample wells.
2. Mix well and incubate at room temperature for 15 minutes.
3. Avoid bubbles while mixing.
4. Measure the absorbance at 575 nm in end point mode.

9. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each standard and sample.
2. Subtract the mean value of the blank (Standard #1) from all readings. This is the corrected absorbance.
3. Plot the corrected values for each standard as a function of the final concentration of Hemoglobin.
4. Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).
5. Apply the corrected sample OD reading to the standard curve to get Hemoglobin (B) amount in the sample wells.
6. Concentration of Hemoglobin in mg/dL in the test samples is calculated as:

$$\text{Sample Hemoglobin Concentration (C)} = B \times D \times 10^* \text{ mg/dL}$$

Where:

B = amount of Hemoglobin in the sample well calculated from standard curve in mg/dL.

D = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up).

* Accounts for sample dilution in the well.

10. FAQs / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

11. Typical Data

Data provided for demonstration purposes only.

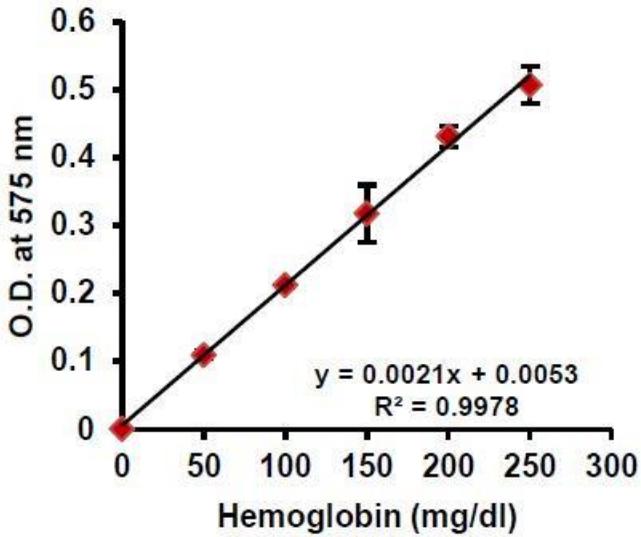


Figure 1. Hemoglobin Standard Curve (0-250 mg/dL).

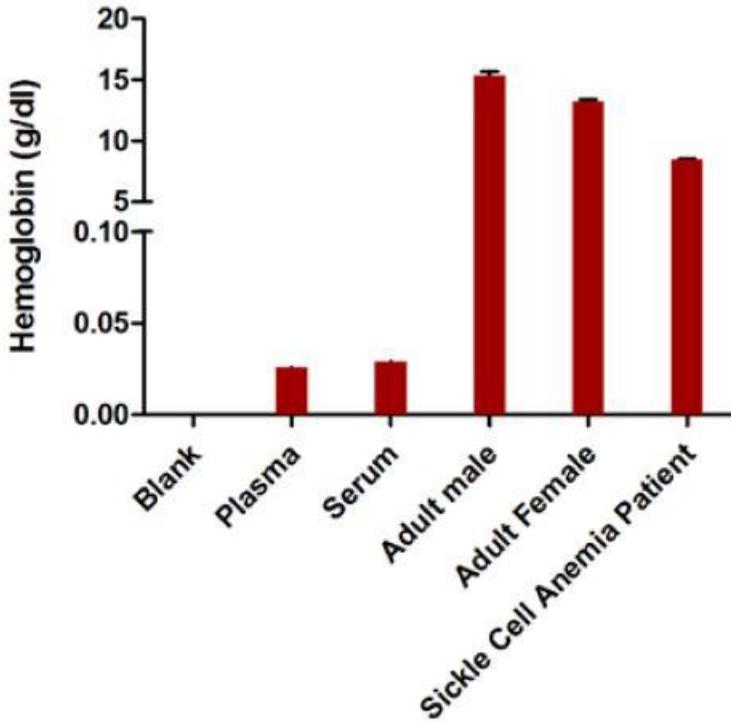


Figure 2. Estimation of hemoglobin concentration in human plasma, serum, adult male, adult female and sickle-cell anemia patient. Whole blood samples were diluted 10-fold. Assays were performed in triplicate following the kit protocol using 20 μ L of the samples.

12. Notes

Technical Support

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