

# Whole Blood Hemoglobin (Hb) Assay Kit (Colorimetric)

LS-K226-250 (250 Tests) • Store at 4°C



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## Introduction

HEMOGLOBIN (Hb) is made of four globin chains each carrying a heme group. It is carried by red blood cells and transports oxygen from the lungs to the peripheral tissues to maintain the viability of cells. Quantitation of blood hemoglobin has been a key diagnostic parameter for various diseases such as anemia, polycythemia and dehydration. Simple, direct and automation-ready procedures for measuring hemoglobin concentration are becoming popular in Research and Drug Discovery. This hemoglobin assay kit is based on an improved Triton/NaOH Method, in which the hemoglobin is converted into a uniform colored end product. The intensity of color, measured at 400 nm, is directly proportional to hemoglobin concentration in the sample. The optimized formulation exhibits high sensitivity and is ideal for measuring hemolysis in low hemoglobin samples (e.g. serum and plasma).

## Key Features

- Sensitive and accurate. Linear detection range 0.9 - 200 mg /dL hemoglobin in 96-well plate assay.
- Simple and high-throughput. The "mix-and-read" procedure involves addition of a single working reagent and reading the optical density. Can be readily automated as a high-throughput assay in 96-well plates for thousands of samples per day.
- Safety. Reagents are non-toxic.
- Versatility. Assays can be executed in 96-well plate or cuvette.

## Applications

- Direct Assays: total hemoglobin in whole blood samples.
- Pharmacology: effects of drugs on hemoglobin metabolism.

## Components

Component	K226-250
	250 Tests
HB Reagent	50 mL
Calibrator (12.0 g/dL)	500 µL

## Materials Not Supplied

Pipetting devices and accessories clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760) or cuvettes and plate reader or spectrophotometer.

## Storage

The kit is shipped at room temperature. Store reagent and calibrator at 4°C. Shelf life: 12 months after receipt.

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**FOR RESEARCH USE ONLY! Not for use in humans.**

LifeSpan BioSciences, Inc. • 2401 Fourth Avenue, Suite 900, Seattle, WA 98121  
[www.LSBio.com](http://www.LSBio.com) • (206) 464-1554 • [TechnicalSupport@LSBio.com](mailto:TechnicalSupport@LSBio.com)

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## Assay Procedure

### Procedure Using 96-Well Plate

Since 5 µL samples are used, when possible perform assays in duplicate or triplicate.

1. Carefully pipette 5 µL water (Blank), 5 µL Calibrator and 5 µL whole blood samples into wells of a clear bottom 96-well plate.
2. Transfer 200 µL HB Reagent into all assay wells. Tap plate to mix.
3. Incubate 5 min at room temperature. Read OD at 570 nm (range 560-600 nm). OD values are stable for at least 60 min.

### Procedure Using Cuvette

1. Pipette 10 µL water (Blank), 10 µL Calibrator and 10 µL whole blood samples into separate cuvettes.
2. Transfer 1 mL HB Reagent into all cuvettes. Mix well.
3. Incubate 5 min at room temperature. Read OD at 570 nm (range 560-600 nm). OD values are stable for at least 60 min.

### Calculations

Subtract blank OD (water) from the Calibrator and Sample OD values. The hemoglobin concentration of Sample is calculated as

$$\text{Hemoglobin} = \frac{OD_{\text{Sample}} - OD_{\text{H}_2\text{O}}}{OD_{\text{Calibrator}} - OD_{\text{H}_2\text{O}}} \times [\text{Calibrator}]$$

$OD_{\text{SAMPLE}}$ ,  $OD_{\text{CALIBRATOR}}$  and  $OD_{\text{H}_2\text{O}}$  are OD values of the sample, the Calibrator (12.0 g/dL) and water.

Conversions: 1.0 g/dL Hb is equivalent to 0.156 mM.

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