# Hemoglobin (Hb)

# Colorimetric Assay kit

# (96 Tests)

Zellbio GmbH (Germany)

CAT No. ZX-44112-96

www.zellx.de

Sample Types Validated for:

Whole blood and RBCs

**!!! Caution: This product is for Research Use Only. Not for *in vitro* Diagnostics!!!**

# Table of Contents



Please read this insert completely prior to using the product.


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# Introduction

## Background

Hemoglobin (Hb) is an erythrocyte protein complex comprised of two sets of identical pairs of subunits, each of which bind an iron-prophyrin group commonly called heme. Generally containing two alpha or alpha-like globulin chains, the remaining subunits may be beta, gamma, delta or epsilon, or in the case of infants, fetal hemoglobin that is replaced during the first year of life. Heme binds and releases oxygen or carbon dioxide in response to slight changes in local gas tension. Free oxygen or carbon dioxide bound by one heme group facilitates subsequent binding by the other heme groups in a given hemoglobin molecule. Subtle changes in pH also regulate hemoglobin affinity for free gases, resulting in a high level of hemostatic control. Hemoglobin values are associated with a variety of conditions ranging from anemias (low Hb), erythrocytosis (high Hb), thalassemia (aberrant chain synthesis), and sickling disorders (abnormal complex shape).

## Assay principle

The ZellX® Hemoglobin Detection kit is designed to quantitatively measure all forms of hemoglobin present in blood and RBCs. The kit uses a single reaction solution that is light stable at 4°C and does not contain dangerous chemicals. All forms of hemoglobin are rapidly converted to a single stable form that is measured photometrically. Many samples can be measured without dilution in this safe, simple assay.

A human hemoglobin standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Standards or diluted samples (10 µL) are pipetted into a clear microtiter plate and the ready-to-use Hemoglobin Detection Reagent is added to each well. The plate is incubated for 30 minutes at room temperature (RT), and then read at 560-580 nm to detect the intensity of the color generated. Results are calculated as g/L for whole blood and RBCs. The concentration of the hemoglobin in the sample is calculated based on the dilution.

# General information

## Materials supplied in the Kit

| *Component* | *Quantity* |
|---|---|
| **Hemoglobin Standard (160 g/L)** | 150 µL |
| **Hb Detection Reagent** | 10 mL |
| **Assay Diluent** | 25 mL |
| **Clear 96 Well Plate** | 1 plate |

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## Storage instruction

All reagents should be stored at 4° C until the expiration date of the kit.

## Materials required but not supplied

Double distilled water (dd$H_2O$)

Microplate/ELISA reader capable of reading optical absorption between 560-580 nm

Precision pipettes, multichannel/repeater pipette and disposable pipette tips

Disposable 1.5-2 mL microtubes for sample preparation

## Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

The Hemoglobin Standard is derived from human blood. It has been extensively tested for viral contamination, but all human blood products should be treated as potentially infectious and adequate precautions taken.

The Hemoglobin Detection Reagent is basic. The solution should not come in contact with skin or eyes. Take appropriate safety precautions when handling this reagent.

Some components of the kit contain sodium azide, which may react with lead or copper plumbing to form potentially explosive complexes. When disposing of reagents always flush with large volumes of water to prevent azide build-up.

## General remarks

- Equilibrate all kit components to room temperature (RT) 30 minutes before use.
- The instruction must be strictly followed.
- The reading of Microplate/ELISA reader must be set at the appropriate wavelength.
- Pipette tips should not be used more than once to prevent cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.

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# Assay protocol

## Sample preparation

Samples containing visible particulate should be centrifuged prior to conducting the assay.

**Whole blood** must be diluted ≥ 1:2 with Assay Diluent prior to conducting the assay.

**RBC samples** should be lysed with Assay Diluent prior to conducting the assay.

**All samples must be used within 2 hours of dilution.**

## Standard preparation

- Prepare a 1:2 dilution of Hemoglobin Standard with Assay Diluent (mix 50 µL of standard with 50 µL of Assay Diluent), and label as the Standard No.6 (80 g/L). Undiluted Standard is used as the standard No. 7 (160 g/L)
- Make series of lower dilutions as described in the table.
- Assay Diluent is used as the 0 g/L standard.

| *No.* | *Concentration* | *Material needed* |
|---|---|---|
| **Standard No.6** | 80 g/L | 50 µL Hb Standard + 50 µL Assay Diluent |
| **Standard No.5** | 40 g/L | 50 µL Standard No.6 + 50 µL Assay Diluent |
| **Standard No.4** | 20 g/L | 50 µL Standard No.5 + 50 µL Assay Diluent |
| **Standard No.3** | 10 g/L | 50 µL Standard No.4 + 50 µL Assay Diluent |
| **Standard No.2** | 5 g/L | 50 µL Standard No.3 + 50 µL Assay Diluent |
| **Standard No.1** | 2.5 g/L | 50 µL Standard No.2 + 50 µL Assay Diluent |
| **Standard No.0** | 0 g/L | 50 µL Assay Diluent |

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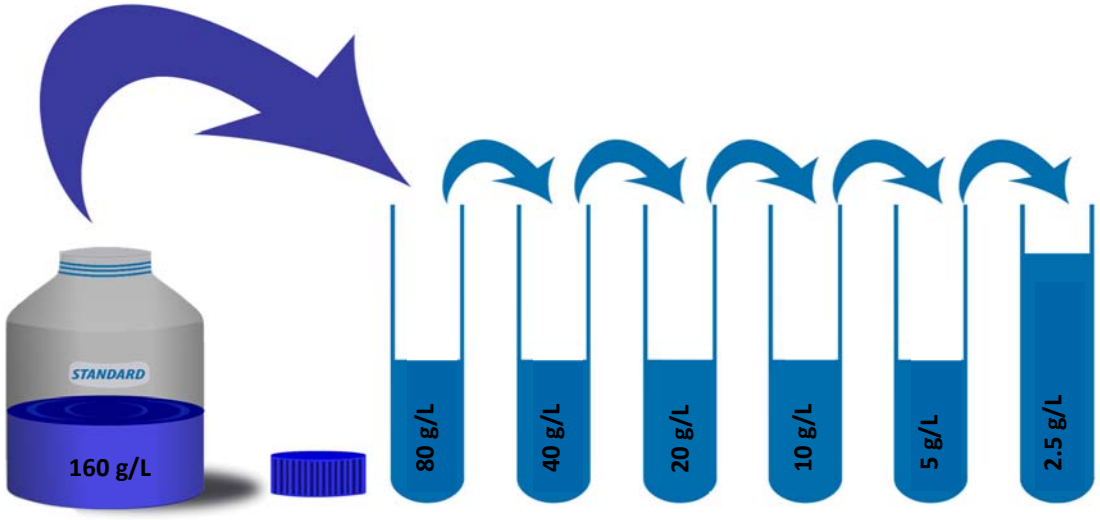


**All standard must be used within 2 hours of preparation.**

## Assay Procedure

1. Pipette 10 µL of either samples or standards into duplicate wells in the plate.
2. Pipette 10 µL of Assay Diluent as the zero standard.
3. Add 100 µL of the Hb Detection Reagent to each well using a multichannel/repeater pipette.
4. Incubate at room temperature for 30 minutes.
5. Read the optical density (OD) at 560-580 nm.

## Calculation

- Average the duplicate OD readings for each standard and sample.
- Subtract the mean ODs for the zero standard from all OD values (for example if the OD value of zero standard, and standard 4 are 0.087, and 1.086 respectively; then the adjusted ODs equal 0 and 0.999 respectively.)
- Create a standard curve by reducing the data using the four parameter logistic curve (4PLC) fitting routine on the plate reader using the adjusted OD values.
- The concentrations obtained should be multiplied by the dilution factor to obtain sample values.

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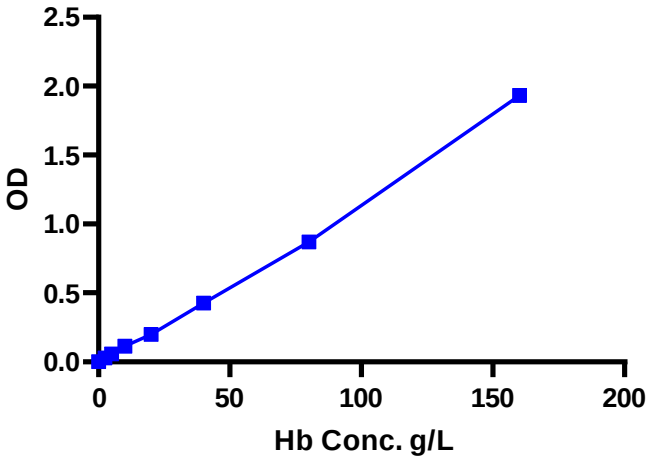


A typical standard curve of ZellX® Hb Assay kit

**Run your own standard curves for calculation of results**

## Assay range

The detection limit of ZellX® Hb assay was determined as 0.21 g/L.

## Sensitivity

The sensitivity of the ZellX® Hb assay was determined as 0.21 g/L.

## Precision

Intra-Assay Precision (Precision within an assay): 3 mammalian samples were tested 20 times in an assay.

Inter-Assay Precision (Precision between assays): 3 mammalian samples were tested in duplicate on 10 different assays over multiple days.

| *Item* | *%CV* |
|---|---|
| **Intra assay** | 3.1, 2.2, 1.7 |
| **Inter assay** | 3.6, 2.9, 7.9 |

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## Interferences

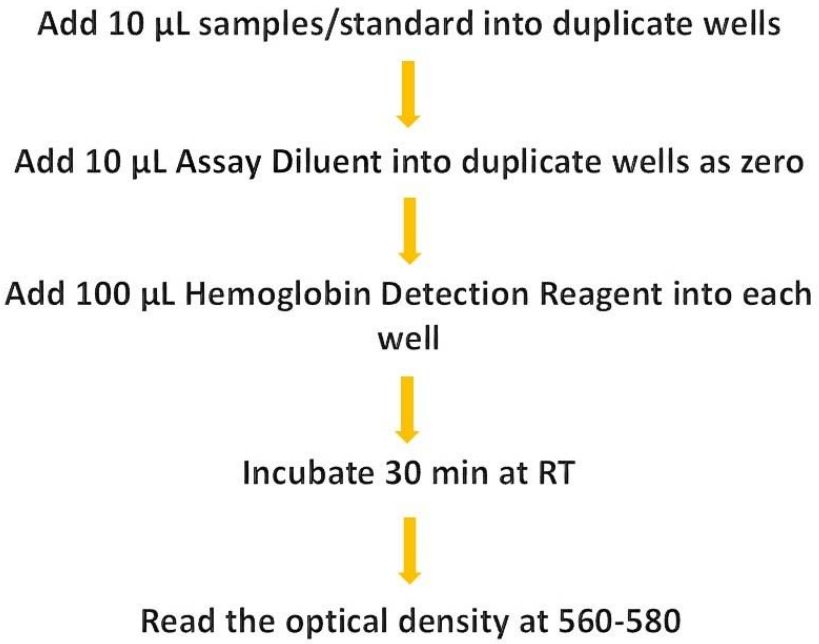
A whole blood sample was serially diluted with 400 g/L BSA to test for protein interference and tested in the assay. No significant change in the measured hemoglobin level was observed over five two-fold dilutions.

To test for glucose interference a whole blood sample was serially diluted with 20 g/L glucose and tested in the assay. No significant change in the measured hemoglobin level was observed over five two-fold dilutions.

For lipid interference a whole blood sample was serially diluted with a mixture containing 8 g/L cholesterol and 112 g/L triglycerides and tested in the assay. No significant change in the measured hemoglobin level was observed over five two-fold dilutions.

A whole blood sample was serially diluted with 20 mg/L of bilirubin and tested in the assay. A 0.7% change in the measured hemoglobin level was observed. Bilirubin at 20 mg/L in normal adults would be considered jaundiced. Newborns can have bilirubin levels above 50 mg/L.

## Protocol summary

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# References


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