# Hemoglobin (Hb) serum & plasma

# Colorimetric Assay kit

# (96 Tests)

Zellbio GmbH (Germany)

CAT No. ZX-44113-96

www.zellx.de

Sample Types Validated for:

Serum and plasma

**!!! 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.


*ZELLX®*
*Krantorweg 48c*
*13503 Berlin*
*Tel.: +49(0)30 81309085*
*Fax: +49(0)30 81309086*
*E-Mail: support@zellx.de*
*Web: www.zellx.de*

# 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 serum and plasma. 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 450 nm to detect the intensity of the color generated. Results are calculated as µg/mL for serum and plasma. 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 (200 µg/mL)** | 45 µL |
| **Hemoglobin Detection Reagent** | 5.5 mL |
| **Assay Buffer Concentrate (5x)** | 5.5 mL |
| **Stop Solution** | 2.5 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

Deionized water (di$H_2O$)

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

Precision pipettes, multichannel/repeater pipettes 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.

The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent

Make sure all buffers used for samples are azide free.

## 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

## Reagent preparation

**Assay Buffer:** Prepare a 1:5 dilution of Assay Buffer Concentrate with diH2O (1 part Assay Buffer Conc. with 4 parts diH2O), and mix well. Assay Buffer can be stored at 4°C for up to 3 months.

## Sample preparation

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

**Serum and plasma samples** must be diluted ≥ 1:20 with Assay Buffer prior to conducting the assay. Further dilution may be needed to ensure that hemoglobin levels for samples fall within the standard curve range

**All samples must be used within 2 hours of dilution; otherwise, keep the sample at -20 or lower temperature.**

## Standard preparation

- Prepare a 1:10 dilution of Hemoglobin Standard with Assay Buffer (mix 10 µL of standard with 90 µL of Assay Buffer), and label as the Standard No.7 (20 µg/mL).
- Make series of lower dilutions as described in the table.
- Assay Buffer is used as the 0 µg/mL standard.

| *No.* | *Concentration* | *Material needed* |
|---|---|---|
| **Standard No.7** | 20 µg/mL | 10 µL Hb Standard + 90 µL Assay Buffer |
| **Standard No.6** | 10 µg/mL | 50 µL Standard No.7 + 50 µL Assay Buffer |
| **Standard No.5** | 5 µg/mL | 50 µL Standard No.6 + 50 µL Assay Buffer |
| **Standard No.4** | 2.5 µg/mL | 50 µL Standard No.5 + 50 µL Assay Buffer |
| **Standard No.3** | 1.25 µg/mL | 50 µL Standard No.4 + 50 µL Assay Buffer |
| **Standard No.2** | 0.625 µg/mL | 50 µL Standard No.3 + 50 µL Assay Buffer |
| **Standard No.1** | 0.3125 µg/mL | 50 µL Standard No.2 + 50 µL Assay Buffer |
| **Standard No.0** | 0 µg/mL | 50 µL Assay Buffer |

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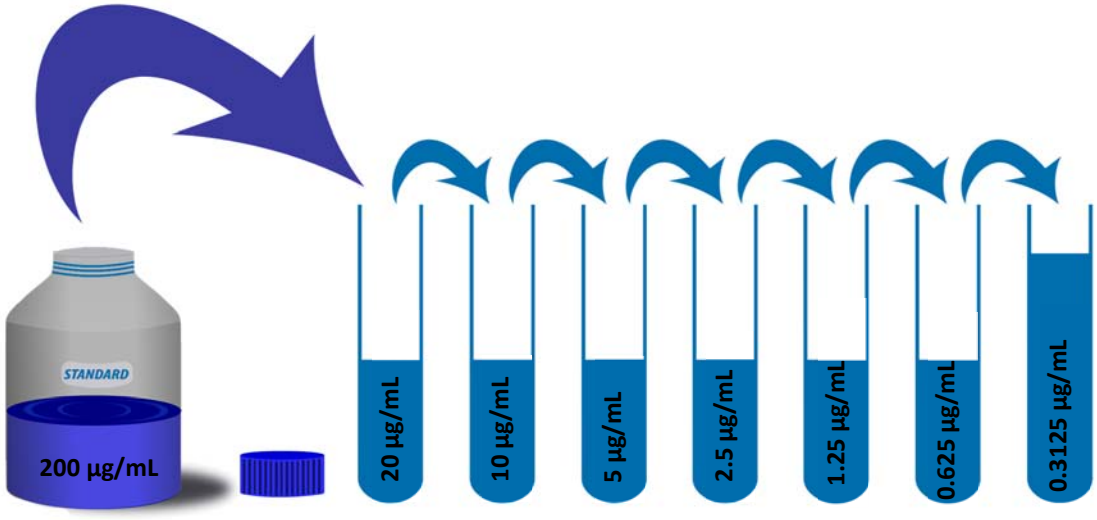


**All standard must be used within 2 hours of preparation.**

## Assay Procedure

1. Pipette 10 µL of either samples or standards into duplicate wells in the plate.
2. Pipette 10 µL of Assay Buffer as the zero standard.
3. Add 50 µL of the Hemoglobin Detection Reagent to each well using a multichannel/repeater pipette.
4. Tap plate gently to mix, and Incubate for 30 minutes at RT.
5. Add 25 µL of Stop Solution to each well using a multichannel/repeater pipette.
6. Read the optical density (OD) at 450 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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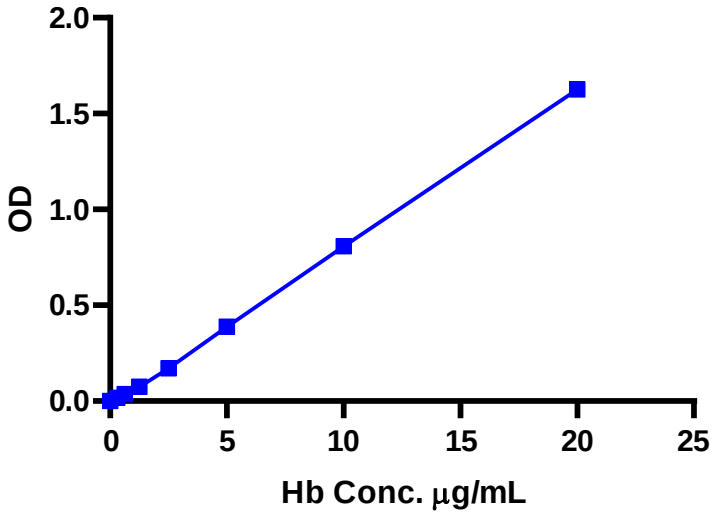


A typical standard curve of ZellX® Hb Assay kit

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

## Assay range

The detection limit of ZellX® Hb assay was determined as 0.082 µg/mL.

## Sensitivity

The sensitivity of the ZellX® Hb assay was determined as 0.053 µg/mL.

## Precision

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

Inter-Assay Precision (Precision between assays): 3 serum samples were tested in duplicate on 19 different assays over multiple days.

| *Item* | *%CV* |
|---|---|
| **Intra assay** | 2.9, 2.8, 2.0 |
| **Inter assay** | 8.7, 9.5, 9.9 |

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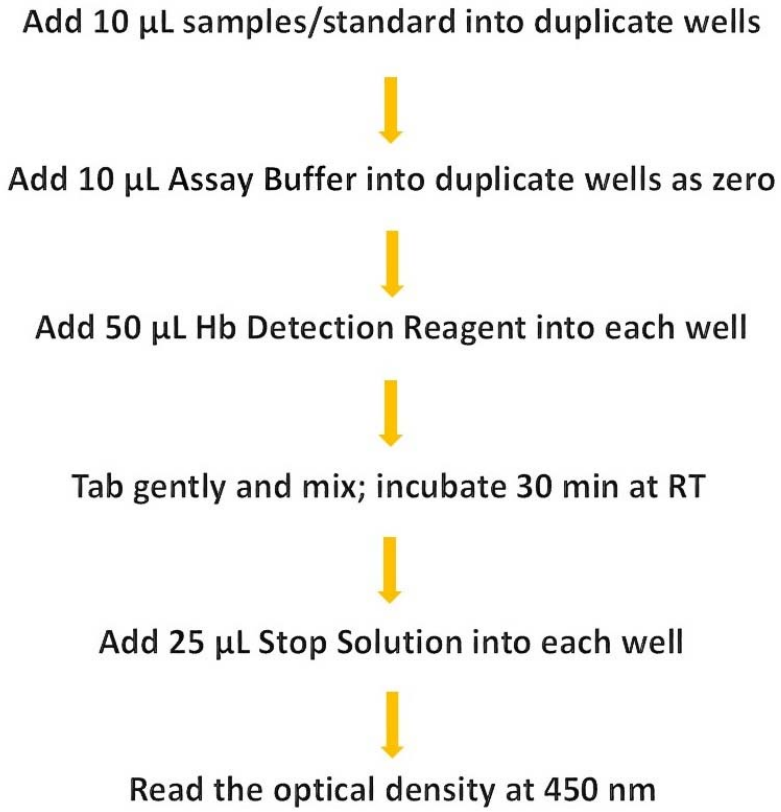
Protocol summary

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# References


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