

## User's Manual and Instructions

### Hemoglobin Assay Kit (Z5030026)

#### Colorimetric Determination of Total Hemoglobin at 400 nm

#### DESCRIPTION

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. BioChain's Hemoglobin Assay Kit is based on an improved cyanohemoglobin 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 substantially reduces interference by substances in the raw samples.

#### APPLICATIONS

**Direct Assays:** total hemoglobin in blood, serum, plasma, urine, etc.

**Pharmacology:** effects of drugs on hemoglobin metabolism.

**Drug Discovery:** HTS for drugs that modulate hemoglobin levels.

#### 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 cuvet.

#### KIT CONTENTS (250 tests in 96-well plates)

**Reagent:** 50 mL

**Calibrator:** 10 mL (equivalent to 100 mg/dL hemoglobin)

**Storage conditions.** The kit is shipped at room temperature. Store reagent and calibrator at 4°C. Shelf life: at least 6 months (see expiry dates on labels).

**Precautions:** reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

#### PROCEDURES

##### Procedure using 96-well plate:

1. **Blank and Calibrator.** Pipette 50  $\mu$ L water (Blank) and 50  $\mu$ L Calibrator into wells of a clear bottom 96-well plate. Transfer 200  $\mu$ L water into the Blank and Calibrator wells.

2. **Samples.** Serum and plasma samples can be assayed directly ( $n = 1$ ). Blood samples should be diluted 100-fold in distilled water ( $n = 100$ ).

Transfer 50  $\mu$ L samples into wells (**Important:** avoid bubble formation during the pipetting steps). Add 200  $\mu$ L Reagent to sample wells and tap plate lightly to mix.

3. Incubate 5 min at room temperature. Read OD at 390-405nm (peak 400nm). Signal is stable for at least 2 hours.

##### Procedure using cuvette:

1. Transfer 100  $\mu$ L sample and 1000  $\mu$ L Reagent into a cuvet and tap lightly to mix. Read OD 400 nm against water.

2. Transfer 100  $\mu$ L Calibrator and 1000 $\mu$ L water to cuvet. Read OD at 400nm against water.

#### CALCULATION

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

$$= \frac{OD_{\text{SAMPLE}} - OD_{\text{BLANK}}}{OD_{\text{CALIBRATOR}} - OD_{\text{BLANK}}} \times 100 \times n \text{ (mg/dL)}$$

$OD_{\text{SAMPLE}}$ ,  $OD_{\text{CALIBRATOR}}$  and  $OD_{\text{BLANK}}$  are OD values of the sample, the Calibrator and water.  $n$  is the dilution factor (100 for blood samples).

**Conversions:** 1mg/dL Hb equals 0.156  $\mu$ M, 0.001% or 10 ppm.

#### MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories.

##### Procedure using 96-well plate:

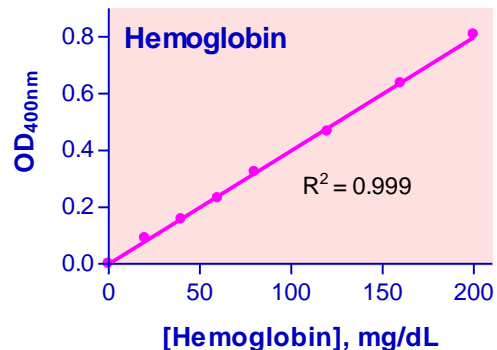
Clear-bottom 96-well plates (e.g. Corning Costar) and plate reader.

##### Procedure using cuvette:

Cuvets and spectrophotometer.

#### EXAMPLES

Hb was determined using the 96-well plate protocol. The values were  $43.4 \pm 0.4$  mg/dL for rat serum,  $11.2 \pm 1.1$  mg/dL for human plasma and  $15.4 \pm 0.7$  g/dL for a mouse whole blood sample.



Standard Curve with Freshly Prepared Hemoglobin  
in 96-well plate assay

#### LITERATURE

[1]. Choudhri TF, Hoh BL, Solomon RA, Connolly ES Jr, Pinsky DJ (1997). Use of a spectrophotometric hemoglobin assay to objectively quantify intracerebral hemorrhage in mice. *Stroke* 28: 2296-2302.

[2]. GREEN P, TEAL CF. (1959). Modification of the cyanmethemoglobin reagent for analysis of hemoglobin in order to avoid precipitation of globulins. *Am J Clin Pathol.* 32:216-217.

[3]. Van Kampen EJ, Zijlstra WG (1965). Determination of hemoglobin and its derivatives. *Adv Clin Chem.* 8:141-187.