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 formula 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
Standard: 1.5 mL 500 mg/dL human hemoglobin
Storage conditions. Store reagent and standard at 4°C. Shelf life: 12 months.
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. Prepare 400 µL 200 mg/dL Premix by mixing 160 µL 500 mg/dL standard and 240 µL distilled water. Dilute standards as follows.

<table>
<thead>
<tr>
<th>No</th>
<th>Premix + H2O</th>
<th>Vol (µL)</th>
<th>Hb (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100µL + 0µL</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>2</td>
<td>80µL + 20µL</td>
<td>100</td>
<td>160</td>
</tr>
<tr>
<td>3</td>
<td>60µL + 40µL</td>
<td>100</td>
<td>120</td>
</tr>
<tr>
<td>4</td>
<td>40µL + 60µL</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>5</td>
<td>30µL + 70µL</td>
<td>100</td>
<td>60</td>
</tr>
<tr>
<td>6</td>
<td>20µL + 80µL</td>
<td>100</td>
<td>40</td>
</tr>
<tr>
<td>7</td>
<td>10µL + 90µL</td>
<td>100</td>
<td>20</td>
</tr>
<tr>
<td>8</td>
<td>0µL + 100µL</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

2. Dilute blood sample 50-fold in water. Transfer 50 µL diluted standards and 50 µL diluted samples into wells of a clear bottom 96-well plate. Important: avoid bubble formation during the pipetting steps.
3. Add 200 µL working reagent and tap plate lightly to mix.
4. Incubate 2 min at room temperature. Read OD at 380-420nm (peak 400nm). Signal is stable for at least 2 hours.

Procedure using cuvette:
1. Prepare standards as for the 96-well plate assay. Transfer 50 µL diluted Standards and 50 µL diluted samples to cuvets.
2. Add 1000 µL working reagent and tap lightly to mix.
3. Read OD at 380-420 nm (peak 400 nm).

CALCULATION
Subtract blank OD (water, #8) from the standard OD values and plot the OD against standard hemoglobin concentrations. Determine the slope using linear regression fitting. The hemoglobin concentration of Sample is calculated as

\[ \text{Hb (mg/dL)} = \left( \frac{\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}}{\text{Slope}} \right) \times n \]

OD_{Sample} and OD_{Blank} are optical density values of the sample and water or buffer in which the sample was diluted, respectively. n is the dilution factor (50 for blood samples).

Conversions: 1mg/dL Hb equals 0.156 mM, 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.

EXAMPLES
Hb was determined using the 96-well plate protocol. The values were 45.1 ± 0.8 mg/dL for rat serum, 10.4 ± 1.2 mg/dL for human plasma and 17.3 ± 0.5 g/dL for a mouse whole blood sample.

LITERATURE

OD_{400nm} m

Hemoglobin

R² = 0.999

Calibration curve in 96-well plate

[|Hemoglobin|, mg/dL]

0.0 0.2 0.4 0.6 0.8

0 50 100 150 200

UK & Rest of World
184 Milton Park, Abingdon
OX14 4SE, Oxford, UK
Tel: +44 (0) 1235 828 200
Fax: +44 (0) 1235 828 482

Switzerland
Centro Nord-Sud 2E
CH-4734 Boggio-Lugano
Tel: +41 (0) 91 604 55 22
Fax: +41 (0) 91 605 17 85

Germany
Suckheimer Landstr. 17/19
60325 Frankfurt/Main
Tel: +49 (0) 69 770099
Fax: +49 (0) 69 13376680

United States
23591 El Toro Rd, Suite 147
Lake Forest, CA 92630
Tel: +1 800 897 0985
Fax: +1 949 265 7703

info@amsbio.com