

# Hemoglobin Assay Kit

Catalog # 6024

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#### **INTRODUCTION**

Hemoglobin is a heme protein carried by erythrocytes and transports oxygen from the lungs to the rest of the body. Therefore low hemoglobin levels in erythrocytes or low erythrocyte numbers in blood deprives the body of oxygen. This condition is defined as anemia. This oxygen deficit can cause dizziness or headaches, while severe or long-lasting anemia can damage organs in the body and even result in death. Anemia is also a complication of various inflammatory diseases such as sepsis (1-3) and autoimmune diseases (4, 5), and has been observed in rodent disease models of inflammatory bowel disease (IBD) (5, 6), colitis (7), sepsis (8), and autoimmune hemolytic anemia (9-12).

As hemoglobin levels correlate to the oxygen carrying-capacity of blood, hemoglobin is a useful marker of anemia to evaluate disease severity and effectiveness of treatments. A standard hemoglobin assay utilizes the toxic cyanmethemoglobin method. However, Chondrex, Inc. would like to introduce a safe, non-cyanide hemoglobin assay kit (13, 14). This assay kit correlates well with the cyanmethemoglobin method and can be used for evaluation of anemia in our mouse sepsis model and mouse IBD model, as well as other disease models. Chondrex, Inc. also provides related products such as FITC-Dextran (Catalog# 4013, 4009), Mouse anti-bacterial antibody ELISAs (Catalog # 6015, 6017, 6019, 6026, 6027, and 6209), and HMGB1 detection kit (Catalog # 6010).

### **KIT COMPONENTS**

Item	Quantity	Amount	Storage
Bovine Hemoglobin Standard (60241)	1 vial	Lyophilized, 2 mg	4°C
Reaction Solution (60242)	1 bottle	20 ml	4°C
ELISA Plate	1 plate	96-well	4°C

#### Figure 1 - Standard Assay layout



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### NOTES BEFORE USING ASSAY

Note: It is recommended that the standards and samples be run in duplicate.

### ASSAY PROCEDURES

 Prepare Standard Dilutions: Reconstitute lyophilized Standard with 1 ml of distilled water to make a 2 mg/ml standard solution. Then serially dilute the 2 mg/ml solution with distilled water. For example, mix 250 µl of the 2 mg/ml solution with an equal volume of distilled water to make a 1 mg/ml solution, and then repeat it five more times to make 0.5, 0.25, 0.125, 0.063, and 0.031 mg/ml solutions. We recommend making fresh serial dilutions for each assay.



2. **Prepare Sample dilutions**: Blood samples should be diluted at 1:200 in distilled water while serum and plasma samples can be assayed directly.

Note: The sample dilution depends on the animal disease models.

- 3. Add Standards and Samples: Use the plate layout as shown in Figure 1. Add 50 µl of standards, distilled water (blank), and diluted samples to wells in duplicate. Pipet carefully to avoid bubbles in the wells.
- 4. Add Reaction Solution: Add 200 µl of Reaction Solution to each well and tap plate lightly to mix. Pipet carefully to avoid bubbles in the wells. Incubate for 15 min at room temperature.
- 5. **Read plate**: Read the plate at 400 nm (390-405 nm).

## CALCULATE HEMOGLOBIN CONCENTRATION

- 1. Average the duplicate OD values for the blank (and sample blank), standards, and test samples.
- 2. Subtract the "blank" (B) values from the averaged OD values of standards and test samples in Step 1
- 3. Plot the OD values of standards against the concentration of hemoglobin (mg/ml). Using a log/log plot will linearize the data. Figure 2 shows a representative experiment where the standard range is from 0.03 2 mg/ml.
- 4. The concentration (mg/ml) of hemoglobin in test samples can be calculated using regression analysis. Multiply the calculated hemoglobin levels by the sample dilution factor to obtain the hemoglobin concentration (mg/ml) in the original sample specimen. Visit www.chondrex. com to download a calculation template for the hemoglobin assay kit.

Conversions: 1 mg/ml hemoglobin equals 100 mg/dl hemoglobin, 15.6 µM, 0.1%, or 1000 ppm.

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Figure 2 - Typical standard curve for hemoglobin assay



Table 1 - Reproducibility of data assayed by the Hemoglobin Assay Kit

Test At	1 mg/ml	0.25 mg/ml	0.063 mg/ml
Inter-Assay CV (%)	4.5	4.2	2.7
Intra-Assay CV (%)	6.3	6.7	5.7
Spiking Test*	104.8%	102.4%	105.2%

Standard was added with known amounts of Hemoglobin and then diluted with distilled water for assaying hemoglobin.

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