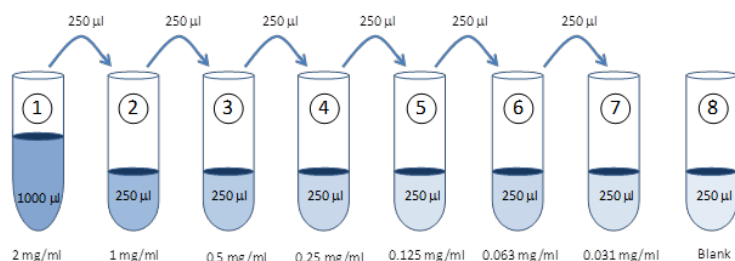


NOTES BEFORE USING ASSAY

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 μ l 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.



- 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.

- Add Standards and Samples:** Use the plate layout as shown in Figure 1. Add 50 μ l of standards, distilled water (blank), and diluted samples to wells in duplicate. Pipet carefully to avoid bubbles in the wells.
- Add Reaction Solution:** Add 200 μ l 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.
- Read plate:** Read the plate at 400 nm (390-405 nm).

CALCULATE HEMOGLOBIN CONCENTRATION

- Average the duplicate OD values for the blank (and sample blank), standards, and test samples.
- Subtract the "blank" (B) values from the averaged OD values of standards and test samples in Step 1
- 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.
- 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 μ M, 0.1%, or 1000 ppm.

Figure 2 - Typical standard curve for hemoglobin assay

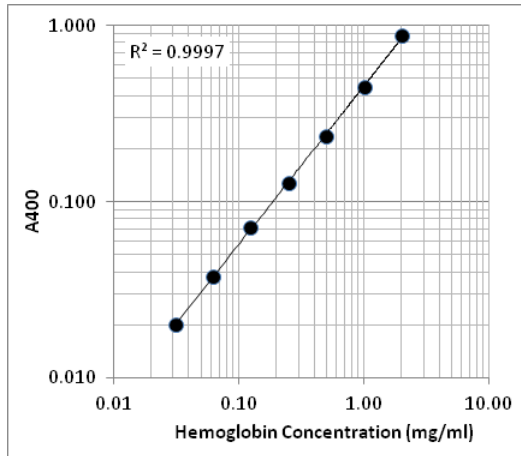


Table 1 - Reproducibility of data assayed by the Hemoglobin Assay Kit

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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