Hemoglobin Colorimetric Detection Kit

Catalog Number EIAHGBC (192 tests)

Rev 1.0

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Hemoglobin Colorimetric Detection Kit is designed to measure all forms of hemoglobin in whole blood, erythrocytes, serum and plasma (EDTA and heparin). For whole blood or erythrocytes samples the assay is performed using the regular format, while for serum and plasma samples the assay is performed using the high sensitivity format.

The assay was characterized with human samples, but can be used to test samples from other species.

Hemoglobin (Hgb) is an erythrocyte protein complex consisting of four globular protein subunits, each of which binds an iron-prophyrin heme group. Hemoglobin generally contains two alpha chains, with the remaining subunits being beta, gamma, delta, or epsilon subunits.

Contents and storage

Kit and components are shipped at –20°C. Upon receipt, store the kit at –20°C. Once open, store the kit at 4°C and use within 2 weeks.

Components	Quantity
Hemoglobin Standard; 16 g/dL hemoglobin from human blood	300 µL
Clear 96-well Plate	2 plates
Hemoglobin Sample Diluent; contains detergent and ≤0.09% sodium azide	50 mL
Hemoglobin Detection Reagent; basic solution, **CAUSTIC**	20 mL

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 560–580 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution

Procedural guidelines

Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.
- The assay is compatible with tissue culture media, but the appropriate media must be selected, as many media contain nitrate salts.
- Lyse erythrocyte/red blood cell (RBC) samples with Hemoglobin Sample Diluent prior to running the assay.

Dilute samples

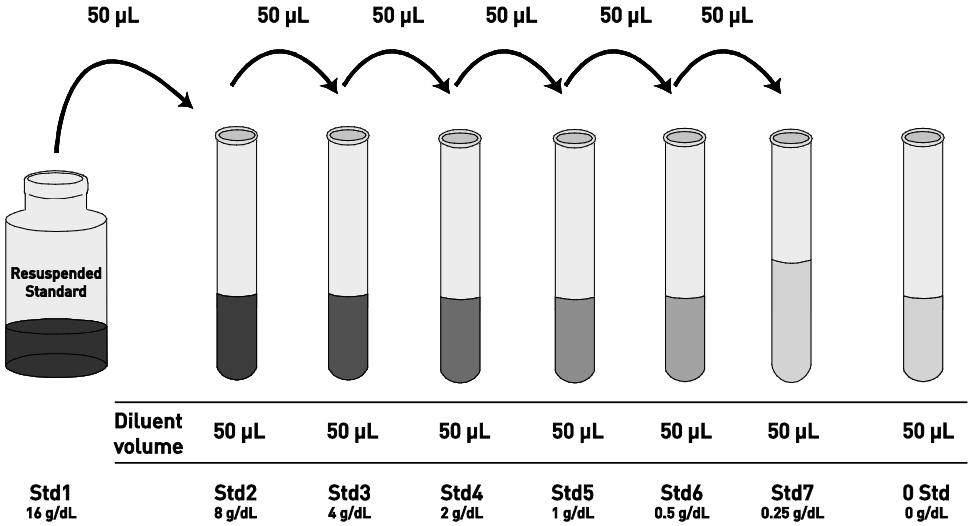
Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

- **Serum** and **plasma** samples can be assayed **without** dilution using the high sensitivity format.
- **Hemolyzed serum and plasma** samples can be assayed **without** dilution using the standard format.
- Perform sample dilutions with Hemoglobin Sample Diluent.
- Dilute **whole blood** samples ≥1:2 in Hemoglobin Sample Diluent.
- Use all samples within **2 hours** of dilution.

Dilute standards (regular format)

Note: Use glass or plastic tubes for diluting standards.

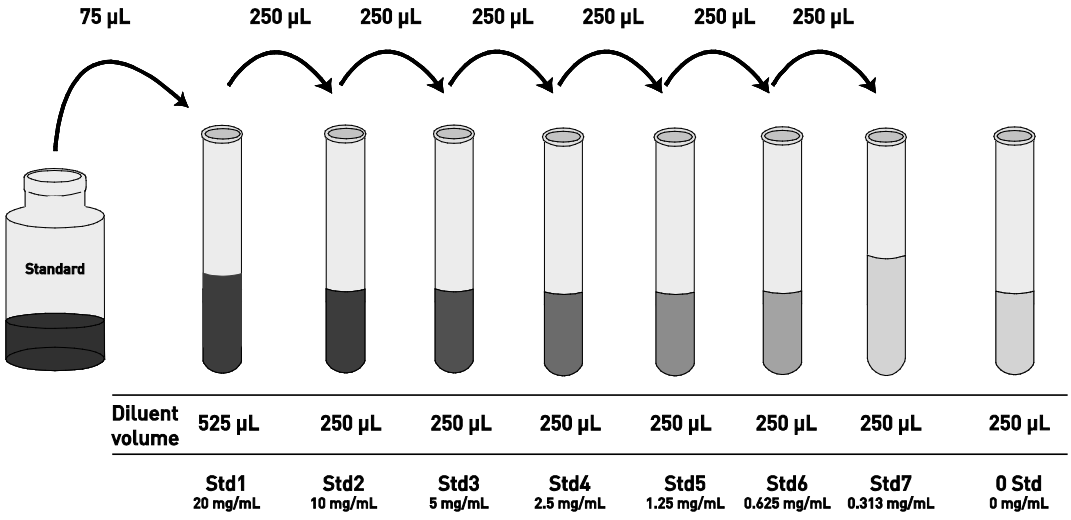
1. Briefly centrifuge the vial of standard to ensure the contents are at the bottom of vial.
2. Add 50 µL Hemoglobin Sample Diluent to each of 7 tubes labeled as follows: 8, 4, 2, 1, 0.5, 0.25, and 0 g/dL hemoglobin.
3. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
4. **Use the standards within 2 hours of preparation**.



Dilute standards (high sensitivity format)

Note: Use glass or plastic tubes for diluting standards.

1. Briefly spin the vial of standard in a microcentrifuge to ensure the contents are at the bottom of vial.
2. Add 75 µL Hemoglobin Standard to one tube containing 525 µL Hemoglobin Sample Diluent and label as 20 mg/mL hemoglobin.
3. Add 250 µL Hemoglobin Sample Diluent to each of 7 tubes labeled as follows: 10, 5, 2.5, 1.25, 0.625, 0.313, and 0 mg/mL hemoglobin.
4. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
5. **Use the standards within 2 hours of preparation**.

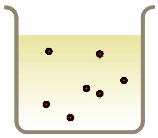


Assay procedure

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 30 minutes.**

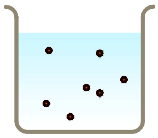
IMPORTANT! Perform a standard curve with each assay.

Regular format



Add sample

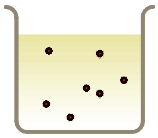
Add 10 µL of standards or diluted samples (see page 2) to the appropriate wells.



Add chromogenic detection reagent

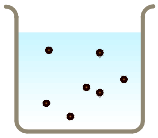
a. Add 100 µL Hemoglobin Detection Reagent into each well.
b. Tap the side of the plate to mix and incubate for 30 minutes at room temperature.

High sensitivity format



Add sample

Add 100 µL of standards or diluted samples (see page 2) to the appropriate wells.



Add chromogenic detection reagent [1]

a. Add 100 µL Hemoglobin Detection Reagent into each well.
b. Tap the side of the plate to mix and incubate for 30 minutes at room temperature.

[1] Bright yellow colored samples can interfere with the high sensitivity format assay, and may require blanking prior to adding Hemoglobin Detection Reagent.

To blank the plate, read the absorbance at 560–580 nm before adding the chromogenic detection reagent. Subtract the absorbance values from the blanking step from the corresponding absorbance measured for each sample.

Read the plate and generate the standard curve

1. Read the absorbance at 560–580 nm.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than that of the highest standard in the appropriate diluent and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards.

Regular format		High sensitivity format	
Standard Hgb (g/dL)	Optical Density (560–580 nm)	Standard Hgb (mg/mL)	Optical Density (560–580 nm)
16	1.993	20	1.978
8	0.870	10	1.019
4	0.426	5	0.508
2	0.199	2.5	0.264
1	0.113	1.25	0.131
0. 5	0.057	0.625	0.068
0.25	0.028	0.313	0.032
0	0.000	0	0.000

Intra-assay precision

Three mammalian samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (g/dL)	7.63	4.73	1.34
%CV	1.7	3.1	2.2

CV = Coefficient of Variation

Inter-assay precision

Three mammalian samples were assayed 10 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (g/dL)	7.48	4.69	1.34
%CV	2.9	3.6	7.9

CV = Coefficient of Variation

Performance characteristics, continued

Expected values

This assay has been tested with whole blood, hemolyzed serum, and plasma (EDTA and heparin) samples from multiple species.

Sample	Range (g/dL)	Average (g/dL)
Human whole blood (n=5) [1]	13.95–21.36	15.77
Human erythrocyte lysates (n=4) [2]	21.58– 40.21	32.23

[1] The normal reference range for human whole blood is 12.0– 17.0 g/dL.
[2] Values not corrected for hemocrits.

Interferents

A variety of substances were tested as possible interfering agents in the assay.

- Protein interference— A whole blood sample was serially diluted with 40 g/dL BSA and tested with the assay. No significant change in the measured hemoglobin level was observed over five two-fold dilutions.
- Glucose interference— A whole blood sample was serially diluted with 2 g/dL glucose and tested with the assay. No significant change in the measured hemoglobin level was observed over five two-fold dilutions.
- Lipid interference— A whole blood sample was serially diluted with a mixture containing 0.8 g/dL glucose and cholesterol and 11.2 g/dL triglycerides, and tested with the assay. No significant change in the measured hemoglobin level was observed over five two-fold dilutions.
- A whole blood sample was serially diluted with 2 mg/dL of bilirubin and tested in the assay. A 0.7% change in the measured hemoglobin level was observed.

Linearity of dilution

Linearity was determined by assaying samples with high and low concentrations of hemoglobin (high sample 9.45 g/dL; low sample 0.46 g/dL) mixed in the ratios shown in the following table.

High Sample %	Low Sample %	Expected Conc. (g/dL)	Observed Conc. (g/dL)	% Recovery
80	20	7.65	7.49	97.9
60	40	5.85	5.62	96.0
40	60	4.05	4.14	102.0
20	80	2.26	2.26	100.3
			Mean Recovery	**99.1%**

Sensitivity

The analytical sensitivity of the assay is 0.021 g/dL hemoglobin for regular format, and 0.020 mg/mL high sensitivity format. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Limited product warranty

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REF Catalog Number | LOT Batch code | Temperature limitation | Use by | Manufacturer | Consult instructions for use | Caution, consult accompanying documents

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