



New
Dual
Range!

Hemoglobin detection kit

Catalog # ADI-907-034

2x96 wells
For use with blood samples



Store at 4°C.



Check our website for additional protocols, technical notes and FAQs.



For proper performance, use the insert provided with each individual kit received.

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Patent Pending

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Introduction

The Hemoglobin Detection kit uses a single reaction solution that is stable at room temperature, not light sensitive and does not contain dangerous chemicals. All forms of hemoglobin are rapidly converted to a single stable form that is measured photometrically. Many samples can be measured without dilution in this safe, simple assay.

Hemoglobin (Hgb) is an erythrocyte protein complex comprised of two sets of identical pairs of subunits, each of which bind an iron-prophyrin group commonly called heme. Generally containing two alpha or alpha-like globulin chains, the remaining subunits may be beta, gamma, delta or epsilon, or in the case of infants, fetal hemoglobin that is replaced during the first year of life. Heme binds and releases oxygen or carbon dioxide in response to slight changes in local gas tension.¹ Free oxygen or carbon dioxide bound by one heme group facilitates subsequent binding by the other heme groups in a given hemoglobin molecule.² Subtle changes in pH also regulate hemoglobin affinity for free gases, resulting in a high level of hemostatic control. Hemoglobin values are associated with a variety of conditions ranging from anemias (low Hgb), erythrocytosis (high Hgb), thalassemias (aberrant chain synthesis), and sickling disorders (abnormal complex shape).¹

The universal reference procedure for hemoglobin determination in blood has been the cyanmethemoglobin method as determined by the Clinical and Laboratory Standards InstituteTM and the International Council for Standardization in Haematology³⁻⁵. In this method, ferricyanide and potassium cyanide convert hemoglobin to a more stable cyanmethemoglobin form that is measured photometrically. While this method is straightforward and uses a single reaction solution, not all forms of hemoglobin are converted to cyanmethemoglobin at the same rate or even to completion. In addition to the safety issues surrounding cyanide, the reagent itself is not stable, so extra care needs to be taken to ensure the quality of any measurement.

Principle

1. Samples and standards are added to the wells of a microtiter plate.
2. Hemoglobin Detection Reagent is added to each well.
3. The plate is incubated at room temperature.
4. Optical density is measured at 570 nm.



Do not mix components from different kit lots or use reagents beyond the kit's expiration date.



The standard should be handled with care due to the known and unknown effects of the antigen.



The physical, chemical, and toxicological properties of the reagents in this kit may not have been fully investigated. Therefore, we recommend the use of gloves, lab coats, and eye protection while using any of these reagents.

Materials Supplied

- 1. Microtiter Plate**
Two plates of 96 wells, Catalog No. 80-2268
The plate is ready to use.
- 2. Hemoglobin Sample Diluent**
50 mL, Catalog No. 80-2270
Sample Diluent containing detergent and $\leq 0.09\%$ sodium azide.
- 3. Hemoglobin Standard**
300 μL , Catalog No. 80-2269
A stock solution of human hemoglobin at 16 g/dL.
CAUTION: From human origin; potentially infectious.
- 4. Hemoglobin Detection Reagent**
20 mL, Catalog No. 80-2271
Special formulation of reagents to detect hemoglobin.
CAUTION: Caustic.

Storage

The kit should be stored at 4°C. All kit components are stable at their recommended storage temperatures until the kit expiration date. Recommended storage temperatures do not necessarily reflect shipping conditions.

Materials Needed but Not Supplied

1. Deionized or distilled water.
2. Precision pipets for volumes between 5 μL and 1,000 μL .
3. Disposable beakers for diluting buffer concentrates.
4. Graduated cylinders.
5. Disposable glass tubes.
6. Microplate reader capable of measuring optical density between 560-580 nm.
7. Software for converting optical density readings from the plate reader and carrying out four parameter logistic curve (4PL) fitting.

Sample Handling

This assay has been validated for whole blood, hemolyzed serum, EDTA plasma, and heparin plasma samples from multiple species. Samples containing visible particulate should be centrifuged prior to using.

Any samples with hemoglobin concentrations above the standard curve range should be diluted further with Hemoglobin Sample Diluent to obtain readings within the standard curve. Use all samples within 2 hours of preparation.

Regular Assay Format

Whole blood must be diluted $\geq 1:2$ with Hemoglobin Sample Diluent prior to running in the kit.

Red blood cells should be lysed with the supplied sample diluent prior to use in this kit.

Hemolyzed serum may be used without dilution.

High Sensitivity Assay Format

Serum and plasma samples may be run without any dilution.

Sample Values

Five human whole blood and four human erythrocyte lysates were tested in the assay. Whole blood values ranged from 21.36 to 13.95 g/dL with an average of 15.77 g/dL and erythrocyte lysates ranged from 21.58 to 40.21 g/dL with an average of 32.23 g/dL, not corrected for hemocrits. Normal reference range for human whole blood is 12.0 – 17.0 g/dL.⁶



Bring all reagents to room temperature for at least 30 minutes prior to opening.

Reagent Preparation

Standard Preparation - Regular Format

Label six glass test tubes as #2 through #7. Briefly vortex and spin the vial of standard in a microcentrifuge to ensure contents are at bottom of vial. The Hemoglobin Standard supplied in the kit is standard 1. Pipet 50 μ L of Hemoglobin Sample Diluent into tubes #2 to #7. Carefully add 50 μ L of the Hemoglobin Standard in the Standard vial provided to tube #2 and vortex completely. Take 50 μ L of the Hemoglobin solution in tube #2 and add it to tube #3 and vortex completely. Repeat the serial dilutions for tubes #4 through #7. The concentration of Hemoglobin in the Hemoglobin Standard vial and tubes #2 through #7 will be 16, 8, 4, 2, 1, 0.5 and 0.25 g/dL.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Assay Buffer Volume (μL)		50	50	50	50	50	50
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Volume of Addition (μL)		50	50	50	50	50	50
Final Conc (g/dL)	16	8	4	2	1	0.5	0.25

Standard Preparation - High Sensitivity Format

Label six glass test tubes as #1 through #7. Briefly vortex and spin the vial of standard in a microcentrifuge to ensure contents are at bottom of vial. Pipet 525 μ L of Hemoglobin Sample Diluent into tube #1, and 250 μ L into tubes #2 to #7. Carefully add 75 μ L of the Hemoglobin Standard provided to tube #1 and vortex completely. Take 250 μ L of the Hemoglobin solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #7. The concentration of Hemoglobin in tubes #1 through #7 will be 20, 10, 5, 2.5, 1.25, 0.625 and 0.313 mg/mL.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Assay Buffer Volume (μL)	525	250	250	250	250	250	250
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Volume of Addition (μL)	75	250	250	250	250	250	250
Final Conc (mg/mL)	20	10	5	2.5	1.25	0.625	0.313

Use all prepared standards within 2 hours of preparation.



Bring all reagents to room temperature for at least 30 minutes prior to opening.

Assay Procedure

Refer to the plate layout at the end of this document to determine the number of wells to be used. Cover unused wells tightly with a plate sealer. **DO NOT REUSE WELLS!**

Regular Format

1. Pipet 10 μ L of Sample Diluent into the zero standard wells.
2. Pipet 10 μ L of standards or samples into the duplicate wells in the plate.
3. Pipet 100 μ L of Hemoglobin Detection Reagent into each well. Gently tap the plate to mix.
4. Incubate for 30 minutes at room temperature.
5. Read fluorescence at 560-590 nm.

High Sensitivity Format

1. Pipet 100 μ L of Sample Diluent into the zero standard wells.
2. Pipet 100 μ L of standards or samples into the duplicate wells in the plate.
3. Pipet 100 μ L of Hemoglobin Detection Reagent into each well. Gently tap the plate to mix.
4. Incubate for 30 minutes at room temperature.
5. Read fluorescence at 560-590 nm.

Calculation of Results

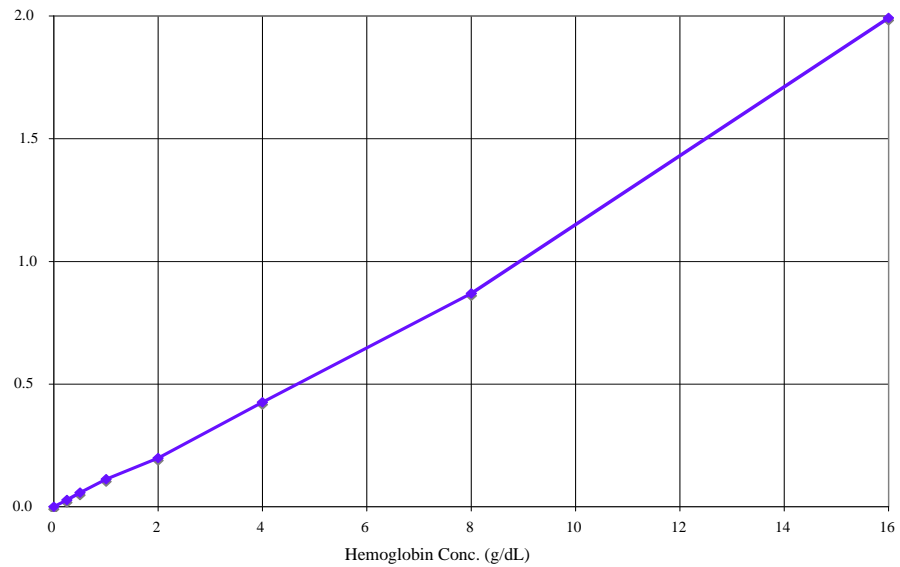
Average the duplicate OD readings for each standard and sample. Subtract the mean OD of the zero standard wells from each standard and sample to get the net OD. Create a standard curve by reducing the net OD using 4PL curve fitting software, such as the Assay Blaster! assay analysis software (Cat. #ADI-28-0002).

Typical Results

This data is for illustration purposes only and should not be used to calculate the results of your experiment.

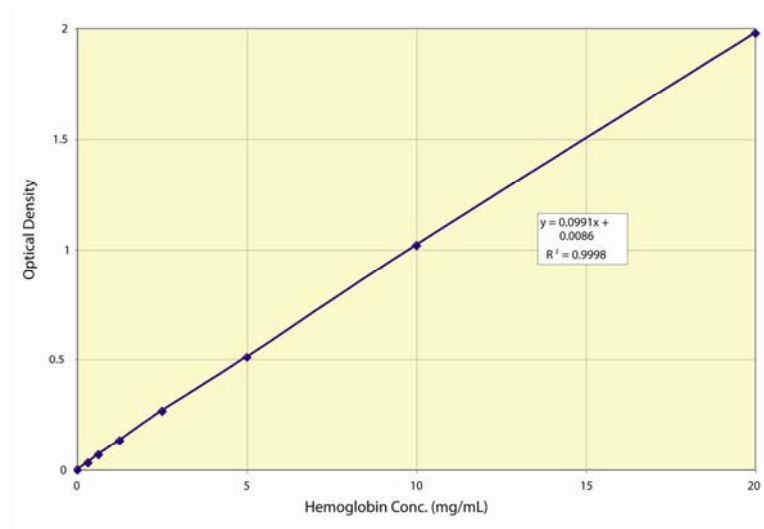
Typical data - Regular Format

Sample	Net OD	Hemoglobin Conc. (g/dL)
Zero	0	0
Standard 1	1.993	16
Standard 2	0.870	8
Standard 3	0.426	4
Standard 4	0.199	2
Standard 5	0.113	1
Standard 6	0.057	0.5
Standard 7	0.028	0.25
Sample 1	0.844	7.64
Sample 2	0.133	1.35



Typical data - High Sensitivity Format

Sample	Net OD	Hemoglobin Conc. (mg/mL)
Zero	0	0
Standard 1	1.978	20
Standard 2	1.019	10
Standard 3	0.508	5
Standard 4	0.264	2.5
Standard 5	0.131	1.25
Standard 6	0.068	0.625
Standard 7	0.032	0.313
Sample 1	1.074	10.56
Sample 2	0.083	0.079



Performance Characteristics

Sensitivity

Sensitivity was calculated by comparing the OD's for twenty wells run for each of the zero and standard #7. The detection limit was determined at two (2) standard deviations from the zero along the standard curve

Sensitivity was determined as 0.0214 g/dL for the Regular Format and 0.0033 g/dL for the High Sensitivity Format.

Limit of Detection

The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty replicates for each of the zero standard and a low concentration diluted human sample.

Limit of Detection was determined as 0.021 g/dL for the Regular format and 0.0033 g/dL for the High Sensitivity format.

Linearity

Serum linearity was determined by taking a human EDTA plasma sample with a low Hgb level of 0.46 g/dL diluted 1:2 with water and a sheep serum spiked with RBCs with a high level of 9.45 g/dL diluted 1:2 with water and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

Low Sample	High Sample	Expected Conc (g/dL)	Observed Conc (g/dL)	% Recovery
100%	0%	0.46	---	---
80%	20%	2.26	2.26	100.3
60%	40%	4.05	4.14	102.0
40%	60%	5.85	5.62	96.0
20%	80%	7.65	7.49	97.9
0%	100%	9.45	---	---
			Mean Recovery	99.1%

Intra Assay Precision

Three mammalian samples were diluted with Hemoglobin Sample Diluent and run in replicates of 20 in an assay. The mean and precision of the calculated hemoglobin concentrations were:

Sample	Hemoglobin Conc. (g/dL)	%CV
1	7.63	1.7
2	4.73	3.1
3	1.34	2.2

Inter Assay Precision

Three mammalian samples were diluted with Hemoglobin Sample Diluent and run in duplicates in ten assays run over multiple days by three operators. The mean and precision of the calculated hemoglobin concentrations were:

Sample	Hemoglobin Conc. (g/dL)	%CV
1	7.48	2.9
2	4.69	3.6
3	1.34	7.9

Interference Data

A whole blood sample was serially diluted with 40 g/dL BSA to test for protein interference and tested in the assay. No significant change in the measured hemoglobin level was observed over five two-fold dilutions.

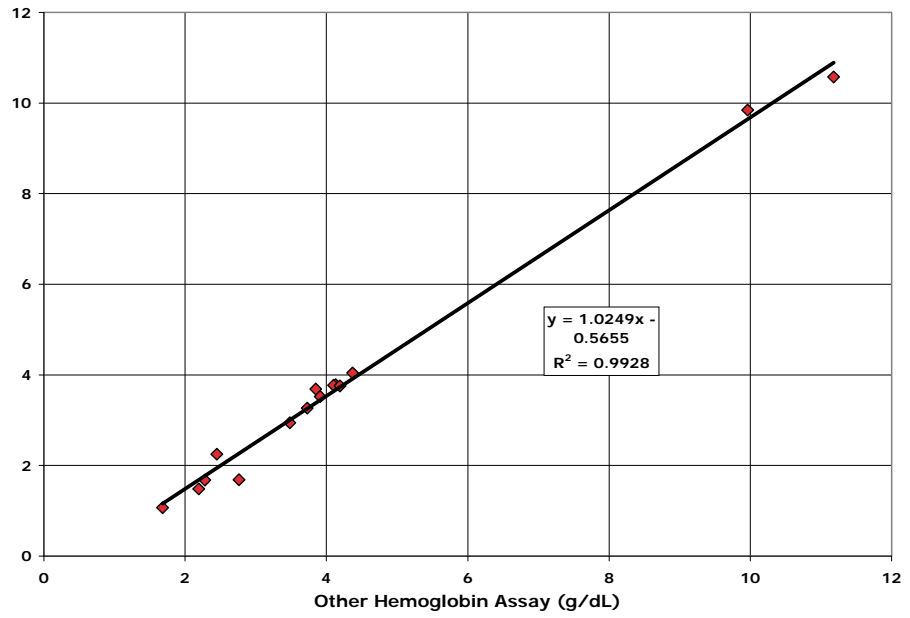
To test for glucose interference a whole blood sample was serially diluted with 2 g/dL glucose and tested in the assay. No significant change in the measured hemoglobin level was observed over five two-fold dilutions.

For lipid interference a whole blood sample was serially diluted with a mixture containing 0.8 g/dL cholesterol and 11.2 g/dL triglycerides and tested in 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. Bilirubin at 2 mg/dL in normal adults would be considered jaundiced. Newborns can have bilirubin levels above 5 mg/dL.

Assay Comparison

15 RBC lysate samples were tested in this kit and in a competitors kit. The correlation result is shown below.



References

1. Tietz, NW, Textbook of Clinical Chemistry, WB Saunders Company, Philadelphia.
2. Manning, JM et al., "Normal and abnormal protein subunit interactions", 1998, J Biol Chem. 273(13):19359-62.
3. Drabkin, DL and JH Austin, "Spectrophotometric Studies: II. Preparations from washed blood cells; nitric oxide hemoglobin and sulf-hemoglobin", 1935, J Biol Chem, 112(1):51-65.
4. Bull, BS, et al., "Reference and selected procedures for the quantitative determination of hemoglobin in blood; approved standard – third edition", NCCLS Vol. 20 No. 28. NCCLS document H15-A3.
5. Rowan, RM, "Recommendations for reference method for haemoglobinometry in human blood (ICSH standard 1995) and specifications for international haemoglobinocyanide standard (4th edition)", 1996, J Clin Pathol, 49:271-74.
6. "Hemoglobin test; Results." MayoClinic.com. 13 July 2009
<http://www.mayoclinic.com/health/hemoglobin-test/MY00529/DSECTION=results>

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Notes

Notes

Use of Product

This product contains research chemicals. As such, they should be used and handled only by or under the supervision of technically qualified individuals. This product is not intended for diagnostic or human use.

Warranty

Enzo Life Sciences International, Inc. makes no warranty of any kind, expressed or implied, which extends beyond the description of the product in this brochure, except that the material will meet our specifications at the time of delivery. Enzo Life Sciences International, Inc. makes no guarantee of results and assumes no liability for injuries, damages or penalties resulting from product use, since the conditions of handling and use are beyond our control.

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