

ARBOR ASSAYS™
Interactive Assay Solutions™



DetectX[®]

Hemoglobin

Colorimetric Detection Kit

2 Plate Kit Catalog Number K013-H1

Species Independent

Sample Types Validated:

Whole Blood and RBCs

Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.

www.ArborAssays.com   

WEB K013-H 240308

TABLE OF CONTENTS

| | |
|---|-------|
| Background | 3 |
| Assay Principle | 4 |
| Related products | 4 |
| Supplied Components | 5 |
| Storage Instructions | 5 |
| Other Materials Required | 6 |
| Precautions | 6 |
| Sample Types | 7 |
| Sample Preparation | 7 |
| Reagent Preparation | 8 |
| Assay Protocol | 9 |
| Calculation of Results | 10 |
| Typical Data | 10-11 |
| Validation Data Sensitivity, Linearity, etc. | 11-13 |
| Sample Values, Interferents and Kit Correlation | 14 |
| Warranty & Contact Information | 15 |
| Plate Layout Sheet | 16 |



BACKGROUND

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 tension¹. Free oxygen or carbon dioxide bound by one heme group facilitates subsequent binding by the other heme groups in a given hemoglobin molecule². 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 Institute™ 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.

1. Tietz, N. W. (1986). Textbook of clinical chemistry. Philadelphia, PA: W. B. Saunders.
2. Manning, J. M., et al. (1998). Normal and abnormal protein subunit reactions. *Journal of Biological Chemistry*, 273(13), 19459–19362.
3. Drabkin, D. L. & Austin, J. H. (1935). Spectrophotometric studies: II. Preparations from washed blood cells; nitric oxide hemoglobin and sulfhemoglobin. *Journal of Biological Chemistry*, 112(1), 51–65.
4. Bull, B. S., et al. (2000) Reference and selected procedures for the quantitative determination of hemoglobin in blood; approved standard—third edition, H15-A3. NCCLS, 20(28).
5. Rowan, R. M. (1996). Recommendations for reference method for haemoglobinometry in human blood (ICSH standard 1995) and specifications for international haemoglobinocyanide standard (4th edition). *Journal of Clinical Pathology*, 49(4), 271–274.

ASSAY PRINCIPLE

The DetectX® Hemoglobin detection kit is designed to quantitatively measure all forms of hemoglobin present in blood and RBCs. Please read the complete kit insert before performing this assay. A human hemoglobin standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Standards or diluted samples are pipetted into a clear microtiter plate and the ready-to-use Hemoglobin Detection Reagent is added to each well. 10 µL of samples and standards are used. The plate is incubated for 30 minutes at room temperature. The plate is read at 560-580 nm to detect the intensity of the color generated. Results are calculated as g/dL for whole blood and RBCs. The concentration of the hemoglobin in the sample is calculated, after making suitable correction for dilution, using software available with most plate readers.

The DetectX® Hemoglobin Detection kit uses a single reaction solution that is light stable at 4°C 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.

RELATED PRODUCTS

| Kits | Catalog No. |
|---|--------------|
| Acetylcholinesterase Fluorescent Activity Kit | K015-F1 |
| Butyrylcholinesterase Fluorescent Activity Kit | K016-F1 |
| Corticosterone ELISA Kits | K014-H1/H5 |
| Cortisol ELISA Kits | K003-H1/H5 |
| Cystatin C ELISA Kit | K012-H1 |
| Glutathione Colorimetric Detection Kit | K006-H1 |
| Glutathione Fluorescent Detection Kits | K006-F1/F5 |
| Glutathione Reductase Activity Kit | K009-F1 |
| Hemoglobin High-Sensitivity Detection Kit | K013-HX1/HX5 |
| Prostaglandin E2 Multi-Format ELISA Kit | K051-H1/H5 |
| Retinol Binding Protein Multi-Format ELISA Kits | K062-H1/H5 |
| Serum Creatinine Detection Enzyme Kit | KB02-H1 |



SUPPLIED COMPONENTS

Clear 96 Well Plates

Corning Costar Plate 9017

Catalog Number X003-2EA

Hemoglobin Standard

A stock solution of human hemoglobin at 16 g/dL.
300 μ L

Catalog Number C037-300UL

Hemoglobin Sample Diluent

Sample diluent containing detergent and \leq 0.09% sodium azide.
50 mL

Catalog Number X052-50ML

Hemoglobin Detection Reagent.

A solution containing chemicals that react with hemoglobin. **CAUSTIC.**
20 mL

Catalog Number C038-20ML

STORAGE INSTRUCTIONS

All components of this kit should be stored at 4°C until the expiration date of the kit.

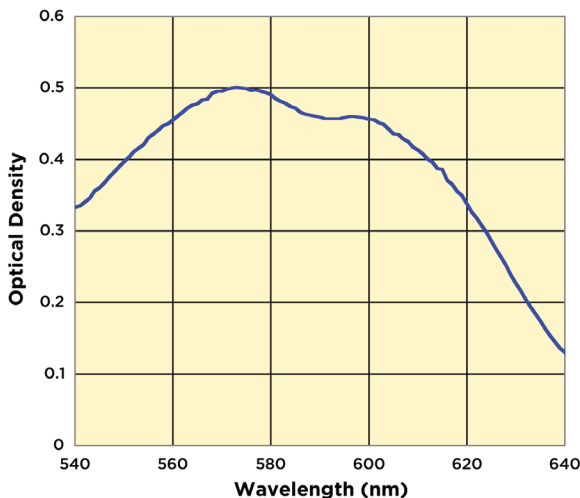
OTHER MATERIALS REQUIRED

Repeater pipet with disposable tips capable of dispensing 100 μ L.

Colorimetric 96 well microplate reader capable of reading optical density at between 560 and 580 nm. Please see spectra of reaction below:

Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

Reaction Spectra



PRECAUTIONS

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

The Hemoglobin Standard is derived from human blood. It has been extensively tested for viral contamination, but all human blood products should be treated as potentially infectious and adequate precautions taken.

The Hemoglobin Detection Reagent is basic. The solution should not come in contact with skin or eyes. Take appropriate safety precautions when handling this reagent.

Some components of the kit contain sodium azide, which may react with lead or copper plumbing to form potentially explosive complexes. When disposing of reagents always flush with large volumes of water to prevent azide build-up.



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SAMPLE TYPES

This assay has been validated whole blood and RBCs from human, chicken and dogfish. Samples containing visible particulate should be centrifuged prior to using.

SAMPLE PREPARATION

Whole Blood

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

Red Blood Cell/Erythrocytes

RBC samples should be lysed with Hemoglobin Sample Diluent prior to running in the kit.

For Serum and Plasma: Use the DetectX[®] Hemoglobin High Sensitivity Detection Kit, K013-HX1/HX5.

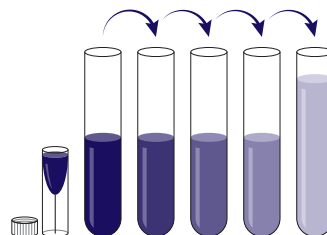
Use all samples within 2 hours of dilution.

REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Standard Preparation

Label 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 μL of Sample Diluent into tubes #2 to #7. Carefully add 50 μL of the Hemoglobin Standard provided to tube #2 and vortex completely. Take 50 μL 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 |
|---|-------|-------|-------|-------|-------|-------|-------|
| Sample Diluent 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 |

Use all Standards within 2 hours of preparation



ASSAY PROTOCOL

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine hemoglobin concentration.

1. Use the plate layout sheet on the back page of the insert to aid in proper sample and standard identification.
2. Pipet 10 μL of samples or standards into wells in the plate. Pipet 10 μL of Sample Diluent into the zero standard wells.
3. Add 100 μL of the DetectX[®] Hemoglobin Detection Reagent to each well, using a repeater pipet. Tap the plate to mix.
4. Incubate at room temperature for 30 minutes.
5. Read the optical density generated from each well in a plate reader capable of reading at 560-580 nm. See spectra on Page 6 for details.
6. Use the plate reader's built-in 4PLC software capabilities to calculate Hemoglobin concentration for each sample.

CALCULATION OF RESULTS

Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean OD's for the Zero standard. The sample concentrations obtained should be multiplied by the dilution factor to obtain neat values.

Or use the online tool from MyAssays to calculate the data:

www.myassays.com/arbor-assays-hemoglobin-colorimetric-detection-kit.assay

TYPICAL DATA

| Sample | Net OD | Hemoglobin Conc. (g/dL) |
|------------|--------|-------------------------|
| Zero | 0 | 0 |
| Standard 1 | 1.695 | 16 |
| Standard 2 | 0.773 | 8 |
| Standard 3 | 0.383 | 4 |
| Standard 4 | 0.181 | 2 |
| Standard 5 | 0.101 | 1 |
| Standard 6 | 0.052 | 0.5 |
| Standard 7 | 0.028 | 0.25 |
| Sample 1 | 0.436 | 4.66 |
| Sample 2 | 0.115 | 1.25 |

Always run your own standard curves for calculation of results. Do not use this data.



*The MyAssays logo is a registered trademark of MyAssays Ltd.

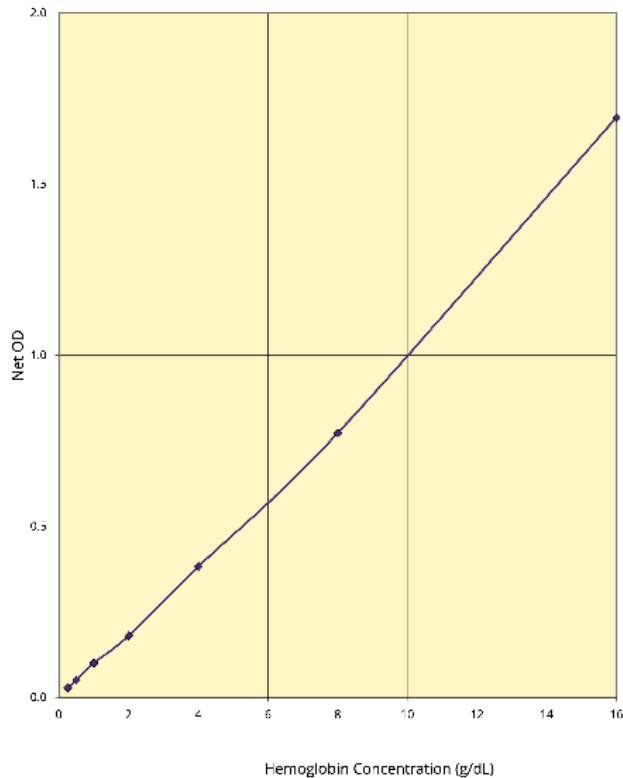
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10

WEB K013-H 240308

EXPECT ASSAY ARTISTRY™

Typical Standard Curves



Always run your own standard curves for calculation of results. Do not use this data.

VALIDATION DATA

Sensitivity and Limit of Detection

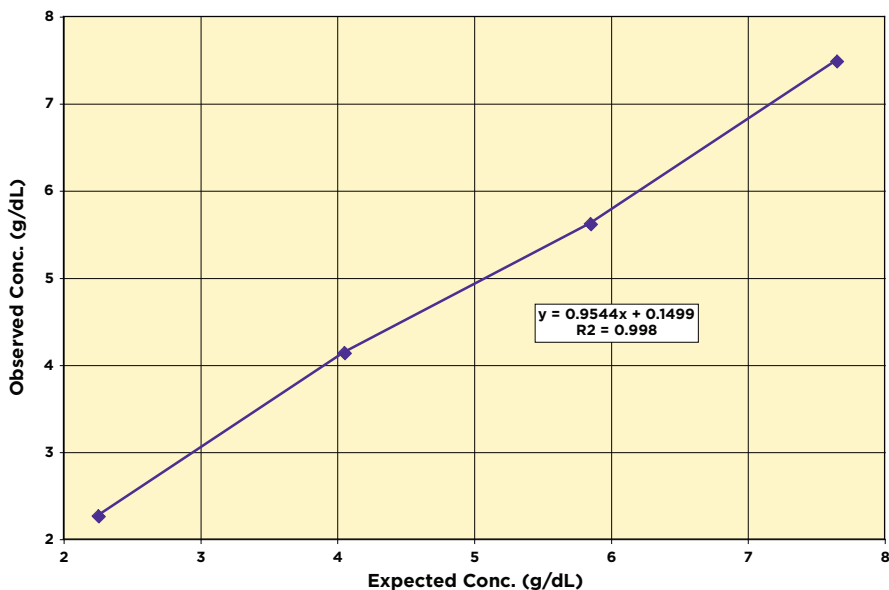
Sensitivity was calculated by comparing the OD's for twenty wells run for each of the zero and standard #7. The detection limit was determined at two (2) standard deviations from the zero along the standard curve. **Sensitivity was determined as 0.021 g/dL.**

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.**

Linearity

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

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

SAMPLE VALUES

This assay has been tested with whole blood and RBC samples from multiple species, including whole blood and RBCs from human, chicken, and dogfish. Five human whole blood and four human erythrocyte lysates were tested in the assay. Whole blood values ranged from 13.95 to 21.36 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/dL⁶.

6. "Hemoglobin test; Results." MayoClinic.com. 13 July 2009
www.mayoclinic.com/health/hemoglobin-test/MY00529/DSECTION=results

INTERFERENTS

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.

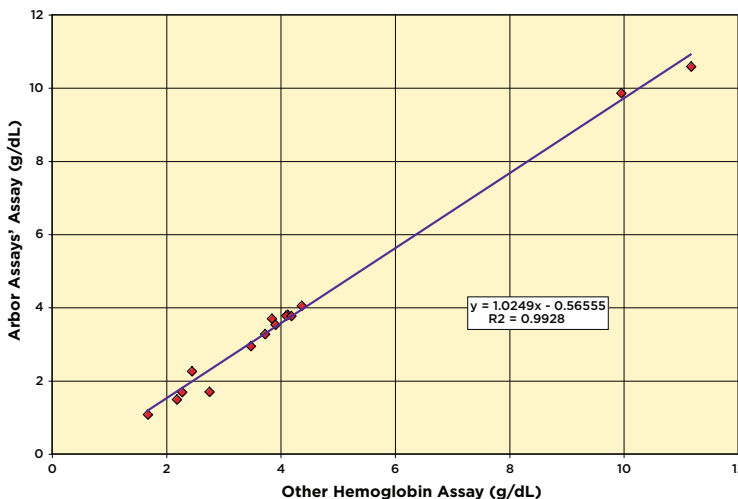
To test for glucose interference a whole blood sample was serially diluted with 2 g/dL glucose and tested in the assay. No significant change in the measured hemoglobin level was observed over five two-fold dilutions.

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.

KIT CORRELATION DATA

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



LIMITED WARRANTY

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

CONTACT INFORMATION

For details concerning this kit or to order any of our products please contact us:

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Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with EIA kits for wildlife conservation research.

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15

WEB K013-H 240308



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ASSAYS

| H | G | F | E | D | C | B | A | |
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