ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES

MOST SENSITIVE

DetectX®

Hemoglobin
High Sensitivity Colorimetric Detection Kit

2 Plate Kit Catalog Number K013-HX1

10 Plate Kit Catalog Number K013-HX5

Species Independent

Sample Types Validated:

Serum and Plasma

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

www.ArborAssays.com

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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 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 cyanmethemoblobin method as determined by the Clinical and Laboratory Standards Institute™ and the International Council for Standardization in Haematology[3-5]. 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 haemiglobinocyanide standard (4th edition). Journal of Clinical Pathology, 49(4), 271–274.

ASSAY PRINCIPLE

The DetectX® High Sensitivity Hemoglobin Detection Kit is designed to quantitatively measure all forms of hemoglobin present in plasma and serum. 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 DetectX® Hemoglobin Detection Reagent is added to each well. The plate is incubated for 30 minutes at room temperature. Stop solution is added at the end of 30 minutes and the plate is read at 450 nm. The concentration of the hemoglobin in the sample is calculated, after making suitable correction for dilution, using software available with most plate readers.

RELATED PRODUCTS

Kits	Catalog No.
Acetylcholinesterase Fluorescent Activity Kit	K015-F1
Butyrylcholinesterase Fluorescent Activity Kit	K016-F1
Corticosterone Enzyme Immunoassay Kits	K014-H1/H5
Cortisol Enzyme Immunoassay Kits	K003-H1/H5 & K003-H1W/H5W
Cystatin C Enzyme Immunoassay Kit	K012-H1
Glutathione Colorimetric Detection Kit	K006-H1
Glutathione Fluorescent Detection Kits	K006-F1/F5
Glutathione Reductase Activity Kit	K009-F1
Glutathione S-Transferase Activity Kit	K008-F1
Hemoglobin Colorimetric Detection Kit	K013-H1
Prostaglandin E2 Multi-Format EIA Kits	K051-H1/H5
Retinol Binding Protein Multi-Format EIA Kits	K062-H1/H5
Serum Creatinine Detection Enzyme Kits	KB02-H1

SUPPLIED COMPONENTS

Clear 96 Well Plates

Bag containing 2 by 96 well plates or 2 bags, each containing 5 by 96 well plates.

Kit K013-HX1 or -HX5	2 or 2 by 5 Each	Catalog Number X003-2EA or -5EA

Hemoglobin Standard

A stock solution of native human hemoglobin at 200 µg/mL.

Kit K013-HX1 **or** -HX5	90 µL **or** 450 µL	Catalog Number C246-90UL **or** -450UL

Assay Buffer Concentrate

A 5X concentrate that must be diluted with deionized or distilled water.

Kit K013-HX1 **or** -HX5	28 mL **or** 55 mL	Catalog Number X067-28ML **or** -55ML

DetectX® Hemoglobin Detection Reagent

Kit K013-HX1 **or** -HX5	11 mL **or** 55 mL	Catalog Number C248-11ML **or** -55ML

Stop Solution

A 1M solution of hydrochloric acid. **CAUSTIC**.

Kit K013-HX1 **or** -HX5	5 mL **or** 25 mL	Catalog Number X020-5ML **or** -25ML

STORAGE INSTRUCTIONS

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

OTHER MATERIALS REQUIRED

Distilled or deionized water.

Repeater pipet with disposable tips capable of dispensing 25 and 50 µL.

Colorimetric 96 well microplate reader capable of reading optical density at 450 nm.

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.

High quality glass or plastic test tubes.

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.

Make sure **all** buffers used for samples are azide free.

The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.

SAMPLE TYPES

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

SAMPLE PREPARATION

Serum and plasma samples must be diluted ≥ 1:20 in the diluted Assay Buffer prior to running the kit. It is up to the end user to determine the appropriate dilution for their samples.

Any samples with hemoglobin concentrations above the standard curve range should be diluted further to obtain readings within the standard curve.

Hemoglobin is stable if stored at 4°C for 7 days. Samples may be frozen and stored at -20°C for long term storage.

Use all samples within 2 hours of preparation or stored at ≤ -20°C until assaying.

REAGENT PREPARATION

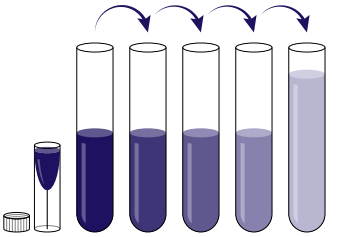
Allow the kit reagents to come to room temperature for 30 minutes. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Assay Buffer

Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted, this is stable at 4°C for 3 months.

Standard Preparation

Label test tubes as #1 through #7. Brie ly vortex and spin the vial of standard in a microcentrifuge to ensure contents are at bottom of vial. Pipet 90 uL of prepared Assay Buffer into tube #1 and 50 µL of prepared Assay Buffer into tubes #2 to #7. Carefully add 10 µL of the Hemoglobin stock solution provided to tube #1 and vortex completely. Take 50 µL 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 µg/mL.



Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Assay Buffer Volume (µL)	90	50	50	50	50	50	50
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Volume of Addition (µL)	10	50	50	50	50	50	50
Final Conc (µg/mL)	20	10	5	2.5	1.25	0.625	0.313

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 Assay Buffer into the zero standard wells.
3. Add 50 µL of the DetectX® Hemoglobin Detection Reagent to each well, using a repeater pipet.
4. Tap plate gently to mix. Incubate at room temperature for 30 minutes.
5. Add 25 µL of the Stop Solution to each well, using a repeater pipet.
6. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
7. 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.

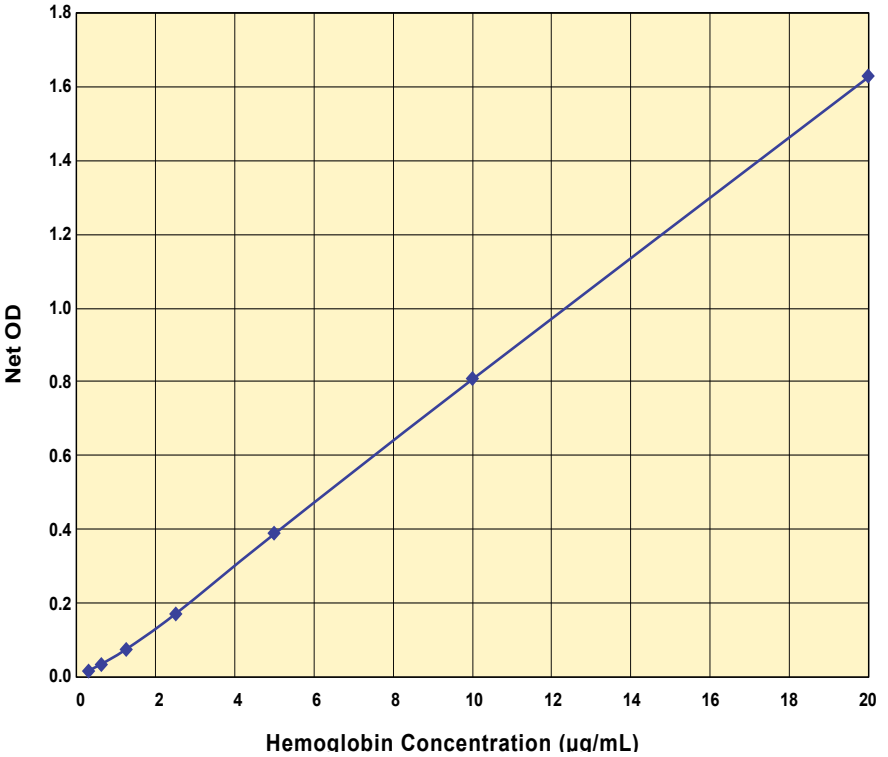
Or use the online tool from MyAssays to calculate the data:
www.myassays.com/arbor-assays-hemoglobin-colorimetric-detection-kit-hs-format.assay

TYPICAL DATA

Sample	Mean OD	Net OD	Hemoglobin Conc. (µg/mL)
Zero	0.049	0	0
Standard 1	1.675	1.626	20
Standard 2	0.856	0.807	10
Standard 3	0.437	0.388	5
Standard 4	0.220	0.171	2.5
Standard 5	0.124	0.075	1.25
Standard 6	0.084	0.035	0.625
Standard 7	0.067	0.018	0.313
Sample 1	0.847	0.798	9.87
Sample 2	0.350	0.301	4.04

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

Typical Standard Curve



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

VALIDATION DATA

Sensitivity and Limit of Detection

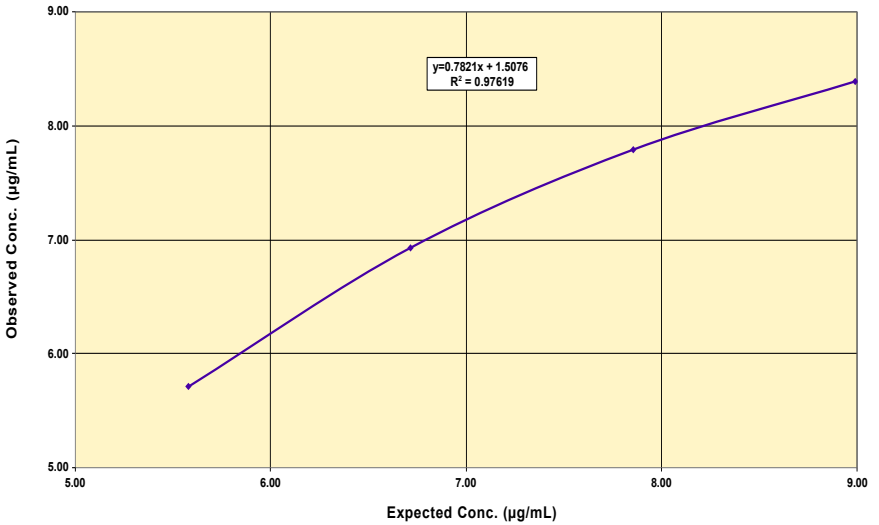
Sensitivity was calculated by comparing the OD's for twenty (20) wells run for each of the zero and standard #7. The detection limit was determined at two (2) standard deviations from the zero along the standard curve. **Sensitivity was determined as 0.053 µg/mL.**

The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty (20) replicates for each of the zero and a low concentration diluted human sample. **Limit of Detection was determined as 0.082 µg/mL.**

Linearity

Linearity was determined by diluting two serum samples, one with a high diluted Hemoglobin level of 10.13 µg/mL and one with a low diluted level of 4.44 µg/mL, mixing them in the ratios given below and running in the assay. The measured concentrations were compared to the expected values based on the ratios used.

High Sample	Low Sample	Expected Conc. (µg/mL)	Observed Conc. (µg/mL)	% Recovery
80%	20%	8.99	8.39	93.3
60%	40%	7.85	7.79	99.2
40%	60%	6.72	6.93	103.2
20%	80%	5.58	5.71	102.4
			Mean Recovery	**99.5%**



Intra Assay Precision

Three serum samples were diluted with Assay Buffer and run in replicates of twenty (20) in an assay. The mean and precision of calculated Hemoglobin concentrations were:

Sample	Hemoglobin Conc. (µg/mL)	%CV
1	11.05	2.8
2	7.45	2.9
3	4.26	2.0

Inter Assay Precision

Three serum samples were diluted with Assay Buffer and run in duplicate in nineteen (19) assays run over multiple days by four operators. The mean and precision of calculated Hemoglobin concentrations were:

Sample	Hemoglobin Conc. (µg/mL)	%CV
1	9.24	8.7
2	6.22	9.5
3	3.77	9.9

SAMPLE VALUES

Thirty-seven (37) human serum and plasma samples from normal, healthy individuals, one of whom was pregnant, were tested in the assay. Neat sample values ranged from 15.6 to 621.6 µg/mL. The normal reference range for serum Hemoglobin concentrations is 120 to 175 µg/mL and for plasma is 0 to 152 µg/mL according to the Mayo Clinic[1].

1. "Test ID: PLHBB Plasma Hemoglobin, Plasma." MayoMedicalLaboratories.com. https://www.mayomedicallaboratories.com/test-catalog/clinical+and+interpretive/9096

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

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OFFICIAL SUPPLIER TO ISWE

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

ASSAYS™
FOR SERIOUS SCIENCE

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