Datasheet for ABIN577655

Hemoglobin Colorimetric Detection Kit

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Overview

Quantity:	2 x 96 tests
Target:	Hemoglobin
Reactivity:	Human, Various Species
Minimum Detection Limit:	0.033 mg/mL
Application:	Biochemical Assay (BCA)

Product Details

Purpose:	The DetectX® Hemoglobin detection kit is designed to quantitatively measure all forms of hemoglobin present in blood and RBCs, or plasma and serum.
Brand:	DetectX®
Sample Type:	Blood, Red Blood Cells, Serum, Plasma
Detection Method:	Colorimetric
Specificity:	Sample Types validated: Whole Blood, RBCs, and hemolyzed Serum and Plasma
Sensitivity:	20 µg/mL
Characteristics:	The Hemoglobin Detection kit uses a single reaction solution that is stable at 4°C, is 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 at 560-580 nm. A human hemoglobin standard is provided to generate a standard curve for the assay. Available as two formats - Regular Format for Whole Blood and RBCs, and a High Sensitivity Format for Plasma & Serum. 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.

Product Details

	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. Free oxygen or carbon dioxide bound by one heme group facilitates subsequent binding by the other heme groups in a given hemoglobin molecule. 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).
Components:	Clear 96 Well Plates Two plates Hemoglobin Standard A stock solution of human hemoglobin at 16 g/dL. 300 µL Hemoglobin Sample Diluent Sample diluent containing detergent and ≤ 0.09% sodium azide. 50 mL Hemoglobin Detection Reagent. A solution containing chemicals that react with hemoglobin. CAUSTiC. 20 mL
Material not included:	Repeater pipet with disposable tips capable of dispensing 100 µL. 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. Reaction Spectra

Target Details

Target:	Hemoglobin
Abstract:	Hemoglobin Products
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),

Target Details

	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 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

Application Details

Application Notes:	This assay has been validated for whole blood, and hemolyzed serum, EDTA and heparin plasma samples from multiple species, including whole blood and RBCs from human, chicken and dogfish. Serum and plasma samples from human, mouse, rabbit and sheep samples were also tested. Samples containing visible particulate should be centrifuged prior to using. Bright yellow colored samples may interfere with the High Sensitivity format and may require blanking prior to addition of the Detection Reagent. Blanking of brightly colored samples is carried out by adding the sample or diluted sample to the plate and reading the optical density at 560- 580 nm beFoRe the addition of the detection reagent. The optical density from this blanking step should be subtracted from the optical density for the samples measured under step 5 on page 9.
Comment:	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 for the Regular format and 0.020 mg/mL (0.0020 g/ dl) for the High Sensitivity format. 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 0.033 mg/mL (0.0033 g/dl) for the High Sensitivity format.
Assay Time:	0.5 h
Protocol:	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. For whole blood or RBC samples 10 µL of samples and standards are used in the Regular format, for serum and plasma samples 100 µL are used in the High Sensitivity format (see page 7). Results are calculated as g/dL for whole blood and RBCs, and mg/mL for serum and plasma. 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. 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.
Reagent Preparation:	Standard Preparation - Regular Format Label 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 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 Standard Preparation - High Sensitivity Format Label 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 µL of Sample Diluent into tube #1, and 250 µL into tubes #2 to #7. Carefully add 75 µL of the Hemoglobin Standard provided to tube #1 and vortex completely. Take 250 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 mg/mL. Std 1 Std 2 Std 3 Std 4 Std 5 Std 6 Std 7 Sample Diluent 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 Standards

	within 2 hours of preparation
Sample Preparation:	For Regular Format Whole blood Whole blood must be diluted ≥ 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 High Sensitivity Format Serum and Plasma Serum and plasma samples should be run in the High Sensitivity format without any dilution. Hemolyzed samples can be read in the Regular format. 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 dilution.
Assay Procedure:	Allow the kit reagents to come to room temperature for 30 minutes. We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine hemoglobin concentration. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit. Regular Format 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. High Sensitivity Format 1. Use the plate layout sheet on the back page of the insert to aid in proper sample and standard identification. 2. Pipet 100 µL of samples or standards into wells in the plate. Pipet 100 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. 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

Application Details

Restrictions:	For Research Use only

Handling

Precaution of Use:	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.
Storage:	4 °C
Storage Comment:	All components of this kit should be stored at 4°C until the expiration date of the kit.

Publications

Product cited in:	Phillips, Geletzke, Smith, Podany, Chacon, Kelleher, Patterson, Soybel: "Impaired recovery from peritoneal inflammation in a mouse model of mild dietary zinc restriction." in: **Molecular nutrition & food research**, Vol. 60, Issue 3, pp. 672-81, (2016) (PubMed). Ammerlaan, Betsou: "Intraindividual Temporal miRNA Variability in Serum, Plasma, and White Blood Cell Subpopulations." in: **Biopreservation and biobanking**, Vol. 14, Issue 5, pp. 390-397, (2016) (PubMed). Buonocore, Grosini, Giardina, Michelotti, Carrabetta, Seneci, Verri, Dossena, Marzatico: " Bioavailability Study of an Innovative Orobuccal Formulation of Glutathione." in: **Oxidative medicine and cellular longevity**, Vol. 2016, pp. 3286365, (2015) (PubMed). Maignan, Briot, Romanini, Gennai, Hazane-Puch, Brouta, Debaty, Ventrillard: "Real-time measurements of endogenous carbon monoxide production in isolated pig lungs." in: **Journal of biomedical optics**, Vol. 19, Issue 4, pp. 047001, (2014) (PubMed).

Publications

Ammerlaan, Trezzi, Lescuyer, Mathay, Hiller, Betsou: "Method validation for preparing serum and plasma samples from human blood for downstream proteomic, metabolomic, and circulating nucleic acid-based applications." in: **Biopreservation and biobanking**, Vol. 12, Issue 4, pp. 269-80, (2014) (PubMed).

There are more publications referencing this product on: Product page

Images

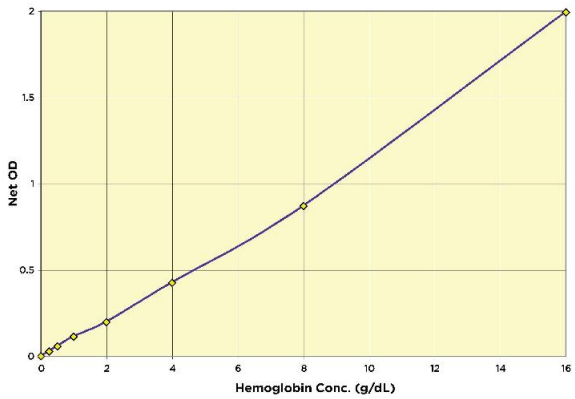

Image 1.

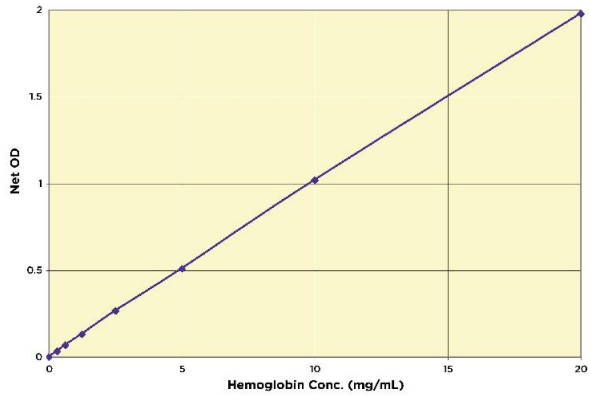

Image 2.