HEMOGLOBIN REAGENT KIT
MD-101106

Intended Use
For the in vitro quantitative measurement of hemoglobin in urine.
NOT FOR USE IN UNPROFESSIONAL SETTINGS.

Summary and Principle
Measuring hemoglobin in urine (also called hemoglobinuria) is an important diagnostic tool because it can indicate underlying health issues related to blood, kidneys, or the urinary tract. Hemoglobin in urine may result from the breakdown of red blood cells (hemolysis) in the bloodstream. It can help diagnose hemolytic anemia, transfusion reactions, or severe infections. Helps distinguish intact red blood cells in urine (hematuria) from free hemoglobin (hemoglobinuria), which has different causes and clinical implications. Free hemoglobin is toxic to kidney tissues. Its presence can signal acute kidney injury (AKI), especially in trauma, burns, or rhabdomyolysis.[1,2,3]
MISSION colorimetric procedure for the determination of free hemoglobin in urine is based upon the peroxidase activity of hemoglobin. In this procedure, hemoglobin activates the oxidation of 3.3', 5.5'-tetramethylbenzidine by hydrogen peroxide to form a chromogenic product with maximum absorption at 650nm. The increase of absorbance is directly proportional to the concentration of hemoglobin in the urine sample. This procedure is simple and accurate and it is based on the work of Lijana, R.C., and Williams, M.C., and Standefer, J.C. and Vanderjagt, D.

Reagents
Reagents are packaged in ready-to-use form. No preparation is required.
Reagent 1 contains: Buffer, 2.3 mmol 3,3',5,5'-tetramethylbenzidine (TMB) and Solubilizer.
Reagent 2 contains: 3 mmol Hydrogen Peroxide and Stabilizer.

Reagents Stability and Storage
Store the unopened MISSION Hemoglobin Kit reagents at 2-8°C. When stored as directed, the reagents are stable until expiration date stated on the label. The Hemoglobin reagents have been tested to reflect shipping conditions and is stable for the lifespan of the product if frozen up to 5 times or reaching temperatures up to 40°C for up to one week. Once opened, the reagents are stable for at least 60 days stored at 2-8°C and capped while not in use.

Cautions
Handle these reagents using good laboratory practice. DO NOT PIPETTE REAGENT BY MOUTH. Avoid contact with skin and eyes. If contact occurs, wash affected area with plenty of cold water. Clean spills immediately. Dispose of in accordance with local regulations and laws. TMB is harmful by inhalation in contact with skin and if swallowed. It is irritating to the eyes. Do not breathe vapor. If contact occurs with eyes, rinse immediately with plenty of cold water. Seek medical attention. TMB is a possible mutagen. Refer to the MSDS for additional information.

Instrumentation
Hemoglobin Reagent Kit are for use on Mission Diagnostics Affirm C200 [1]and Beckman AU680 Analyzers. Refer to instrument procedure instructions in the instrument manual provided with the specific analyzer.

Specimen Collection and Handling
Sample collection: midstream, random urine in sterile container. Transport samples within 2 hours at room temperature to the laboratory.
Storage: 2 hours at room temperature. Refrigerate at 2–8°C (stable up to 24 hours).
Avoid freezing, contamination, delayed analysis.

Quality Control
Store and handle reagents properly before and during use. Every laboratory should establish its own test requirements using controls and calibrators. MISSION Diagnostics provides Calibrators to meet your program needs and which conform to NLCP Guidelines[4]:

MD-101206 – Hemoglobin Calibrator

Specificity, Limitations, and Interferences
Menstrual blood contamination, strong oxidizing agents (e.g., some cleaning products in containers) and dehydration or concentrated urine increasing apparent hemoglobin can lead to false positive results. Diluted urine may lower hemoglobin concentration below detection. Delayed testing or improper storage can lead to hemoglobin degradation.

Typical Performance Characteristics
The following performance data was obtained using the Affirm C200 and Beckman AU680 Analyzers. Other instruments may yield different performance data.

[1] Also known as Zybio EXC200 Analyzer

Distributed by: Mission Diagnostics LLC, 1 Burton Dr., Meridith, NH 03246
Manufactured by: Diamond Diagnostics Inc., 333 Fiske St., Holliston, MA 01746
Technical Assistance Call: 1.508.429.0450
Email: support@diamonddiagnostics.com

Linearity
The following results were obtained on an Affirm C200 and Beckman AU680 Analyzers using the MISSION Hemoglobin Reagent Kit on samples containing 0.25, 0.5, 1.0, 2.5 and 5.0 mg/dL hemoglobin. The table below includes mean, standard deviation (SD) and Coefficient of Variation (CV) for each value.

Mean (mg/dL)	SD	CV%
0.28	0.02	5.6
0.52	0.05	10.0
1.08	0.04	3.9
2.53	0.04	1.6
4.83	0.09	1.8

Precision
Studies performed on Affirm C200 and Beckman AU680 Analyzers. The precision of the assay was evaluated following a modification of NCCLS protocol EPT-T2. The within-run precision data was obtained by running two samples in replicates of 20 on the same day. The run-to-run data was obtained by running two samples in replicates over a five-day period.

Within-Run			Run-to-Run		
Mean (mg/dL)	SD	CV%	Mean (mg/dL)	SD	CV%
9.676	0.213	2.2	9.02	1.27	14.1
38.758	1.096	2.8	36.55	3.22	8.8

Analytical Specificity
Analytical Specificity Cross contamination studies have not been performed on Affirm C200 and Beckman AU680 Analyzers. Certain reagent/ instrument combinations used in sequence with this assay may interfere with reagent performance and test results. The existence of, or effects of, any potential cross contamination issues are unknown.

Test Conditions
For the data presented in this insert, studies using this reagent were performed on Affirm C200 and Beckman AU680 Analyzers using the parameters listed below.

Limitations
A sample with a hemoglobin concentration exceeding the analytical measuring range should be diluted with deionized water and reanalyzed incorporating the dilution factor in the calculation of the value.

Calibration
Calibration material should be used to calibrate the procedure. The frequency of calibration using an automated system is dependent on the system and the parameters used. If control results are found to be out of range, the test may need to be re-calibrated. Under typical operating conditions manufacturer calibration stability studies have shown the calibration curve will be stable for at least 14 days.

Method Parameters

Analyzer Specific Settings

Method type:	Endpoint
Slope:	positive
Units	mg/dL
DOM wavelength	660
SUB wavelength	750
Sample volume	2
R1 volume	200
R2 volume	80
Blank rxn read (cycles)	16 - 20
Sample rxn read (cycles)	36 - 40

Calibration Settings

Calibration Type	Linear
Reagent Blank required	Yes
Calibrator 1	1
Calibrator 2	5

Analytical Measuring Range (AMR)

Range: (low)	0
Range: (high)	10

BIBLIOGRAPHY
1. Rifai, N., Horvath, A. R., & Wittwer, C. T. (Eds.). (2017). *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics* (6th ed.). Elsevier.
2. McPherson, R. A., & Pincus, M. R. (Eds.). (2022). *Henry's Clinical Diagnosis and Management by Laboratory Methods* (24th ed.). Elsevier.
3. McPherson & Pincus: Henry's Clinical Diagnosis and Management by Laboratory Methods.
4. National Laboratory Certification Program. General Laboratory Inspection Checklist / Report. OMB No. 0930-0158. November, 2002.

Distributed by: Mission Diagnostics LLC, 1 Burton Dr., Meridith, NH 03246
Manufactured by: Diamond Diagnostics Inc., 333 Fiske St., Holliston, MA 01746
Technical Assistance Call: 1.508.429.0450
Email: support@diamonddiagnostics.com