CATACHEM

Plasma Free Hemoglobin (PFH) Reagent Kits

V464-0B

IVDD, MHRA, CE

In the USA, for research use only and not for use in diagnostic procedures.

CONTENTS	PRODUCT NO.	PACKAGING
PLASMA FREE HEMOGLOBIN KIT	**V464-0B**	
PFH SUBSTRATE REAGENT (R1)	V464-03	2 X 12.5 mL
PFH ACTIVATOR REAGENT (R2)	V464-04	2 x 5.0 mL
PFH CALIBRATOR	V464-20	1 x 3 mL
PFH CONTROL LEVEL I	V464-21	1 x 3 mL
PFH CONTROL LEVEL 2	V464-22	1 x 3 mL

CALIBRATOR AND CONTROLS

PFH CALIBRATOR **STORAGE:** 2-8°C **LOT NO:** XX000000
V464-20 (1 x 3 mL) **EXPIRATION DATE:** YYYY-MM-DD

CONSTITUENT	METHOD PRINCIPLE	CALIBRATION VALUE CONVENTIONAL UNITS	CONVENTIONAL UNITS	CALIBRATION VALUE S.I. UNITS	S.I. UNITS
Plasma Free Hemoglobin Calibrator	Kinetic Rate UV Method	25	mg/dL	25	mmol/L

PFH CONTROL LEVEL 1 **STORAGE:** 2-8°C **LOT NO:** XX000000
V464-21 (1 x 3 mL) **EXPIRATION DATE:** YYYY-MM-DD

CONSTITUENT	METHOD PRINCIPLE	CALIBRATION VALUE CONVENTIONAL UNITS		CONVENTIONAL UNITS	CALIBRATION VALUE S.I. UNITS		S.I. UNITS
		Mean	Range		Mean	Range	
Plasma Free Hemoglobin Control Level 1	Kinetic Rate UV Method	5.0	2.0 – 8.0	mg/dL	5.0	2.0 – 8.0	mmol/L

PFH CONTROL LEVEL 2 **STORAGE:** 2-8°C **LOT NO:** XX000000
V464-22 (1 x 3 mL) **EXPIRATION DATE:** YYYY-MM-DD

CONSTITUENT	METHOD PRINCIPLE	CALIBRATION VALUE CONVENTIONAL UNITS		CONVENTIONAL UNITS	CALIBRATION VALUE S.I. UNITS		S.I. UNITS
		Mean	Range		Mean	Range	
Plasma Free Hemoglobin Control Level 2	Kinetic Rate UV Method	50	35 – 65	mg/dL	50	35 – 65	mmol/L

Assigned values, listed in the above tables, were obtained from multiple assays over a period of seven days on an automated analyzer. Verify that the product lot number on the data sheet corresponds to the lot number printed on the vial label. Plasma Free Hemoglobin Calibrator and Controls contain serum. This kit is non-sterile and single use.

INTENDED USE

The Catachem Plasma Free Hemoglobin Reagent Kit is intended for in vitro diagnostics use in the manual or automated, quantitative, determination of Plasma Free Hemoglobin in serum or plasma. This kit is intended for use in near-patient testing.

REAGENT PREPARATION

No preparation is required. The Catachem Plasma Free Hemoglobin reagents, calibrators, and controls are liquid and ready to use.

Instructions for use

1. Bring the liquid, ready-for-use Catachem PFH Calibrator, Controls Level 1 and Level 2 to room temperature (15-30°C). Tilt the bottle to mix gently but thoroughly.
2. Proceed with use, following the instructions of the assay procedure and as directed by instrument manufacturer.
3. Following use, tightly cap vials and store unused material refrigerated between 2-8°C.
4. Dispose of materials in accordance with all applicable local and national regulations. Take care to align with your employer's chemical-specific and universal/standard precautions.

Reagent Storage and Stability

Once the Plasma Free Hemoglobin Substrate Reagent (R1) and Plasma Free Hemoglobin Activator Reagent (R2) have been opened, they are stable at 2-8°C (refrigerated) for at least 60 days if tightly capped when not in use. Store the unopened Catachem Plasma Free Hemoglobin reagents, calibrator, and controls at 2-8°C (refrigerated). When stored as directed, the products are stable until the expiration date stated on the label. Once opened, the Catachem Plasma Free Hemoglobin Calibrator and Controls Level 1 and Level 2 are stable for at least 60 days at 2-8°C. Products should be capped tightly between uses. Erroneous results may occur from prolonged exposure of opened vial to ambient air and/or elevated temperatures. Reagent or analyzer modifications may give a value other than that listed in assay data section.

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Summary

The use of specific calibrators and assayed quality control materials is an established procedure for optimal performance of manual or automated assay systems and is part of good laboratory practice. The Catachem Plasma Free Hemoglobin Calibrator, Control Level 1 and Level 2 are liquid, ready to use products that have been specifically formulated for use with the Catachem Plasma Free Hemoglobin Assay Method.

Intended Purpose

In the United States, for **research use only and not for use in diagnostic procedures.** NOT FOR USE IN UNPROFESSIONAL SETTINGS. For use in the automated, quantitative determination of Plasma Free Hemoglobin in serum or plasma. Catachem Plasma Free Hemoglobin Calibrator and bi-level Controls are intended for use in calibration and as assayed quality control material. These materials are to be used with the Catachem Plasma Free Hemoglobin manual or automated quantitative assay of serum or plasma in accordance with the instrument manufacturer's directions.

Clinical Significance (1-2)

Measurement of Plasma Free Hemoglobin is of value in monitoring hemolytic transfusion reaction and certain other conditions associated with intravascular hemolysis. An increase in Plasma Hemoglobin is indicative of acute destruction of erythrocytes (hemolysis) within the vascular system as well as intravascular destruction (hemolysis) of red blood cells. It is used by clinicians to evaluate hemolytic anemia, especially intravascular hemolysis. Plasma Hemoglobin is increased with intravascular hemolysis, ABO incompatible transfusion, traumatic hemolysis, falciparum malaria, burns, and march hemoglobinuria. Increase may occur in some cases of extra vascular hemolysis, delayed transfusion reaction, slight increase in sickle cell anemia, and β-thalassemia.

Method Principle (3-4)

Catachem's colorimetric procedure for the determination of free hemoglobin in plasma 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 in absorbance is directly proportional to the concentration of hemoglobin in the plasma 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 Vanderjaagt, D.

Reagents

Substrate Reagent
Each liter contains:

Buffer	
3,3',5,5'-tetramethylbenzidine (TMB)	2.3 mmoles
Solubilizer	

Activator Reagent
Each liter contains:

Hydrogen Peroxide	3.0 mmoles
Stabilizer	

Precautions

Handle these materials with gloves and personal protective equipment according to good laboratory practices as employed when handling live samples or any biological sample. Avoid contact with skin and eyes. If contact occurs, wash affected area with plenty of cold water. Contain and clean spills immediately. Dispose of materials according to local regulations and laws. Refer to SDS for additional information and determination of any residual risks. Calibrator, controls, and linearity materials contain tissues.

TMB is harmful by inhalation, if in contact with the skin, or 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.

Reagent Indications of Deterioration

- Turbidity
- Absorbance > 0.5 OD, 1 cm light path, 650 nm
- Quality control values out of assigned ranges

If these reagent characteristics are observed, contact Catachem technical service.

Specimen Collection and Stability (6)

Clear non-hemolyzed plasma collected in heparin as anticoagulant is the specimen of choice. Blood should be drawn without trauma and handled carefully to avoid hemolysis. Hemolysis may occur if blood is drawn too quickly into the collection tube or subjected to vigorous mixing. Testing should be done promptly after specimen collection. If testing is delayed, store plasma specimen, removed from the blood cells, frozen at -20°c. Specimens collected with anticoagulants such as EDTA, Oxalate, and other should not be used since they are known to interfere with the hemoglobin-peroxide reaction.

Materials Provided

Catachem Plasma Free Hemoglobin Substrate Reagents
Catachem Plasma Free Hemoglobin Activator Reagents

Materials Required but Not Provided

- Spectrophotometer equipped with 650nm wavelength.

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- Calibrator material with assigned Plasma Free Hemoglobin values.
- Quality control materials with assigned Plasma Free Hemoglobin values.
- Cuvettes (1 cm light path)
- Timer (to time incubation time)
- Pipette (for reagents)
- Pipette (for sample)

Calibration
Catachem's Plasma Free Hemoglobin Calibrator, product no. V464-20, which has a known Plasma Hemoglobin value, is recommended.

Calibration Schedule
Calibration should be performed when this method is implemented for the first time and when quality control values fall outside of the indicated range.

Calibration Procedure
The Catachem Plasma Free Hemoglobin Calibrator is utilized by the analyst to check calibration of a manual or automated Catachem Plasma Free Hemoglobin reagent system in accordance with the instrument manufacturer's directions. Instructions for calibrating the automated analyzer/spectrophotometer are provided by the specific instrument manufacture. Read the entire recommended calibration procedure before proceeding with the instrument calibration.

Quality Control
To monitor the quality performance of the procedure, Catachem recommends the use of Catachem Plasma Free Hemoglobin Control Level 1 and Level 2, product no. V464-21 and V464-22, with assigned Plasma Free Hemoglobin values. These quality control materials should, if feasible, be included in the assay each time the procedure is performed.

Directions for Use
Catachem's Plasma Free Hemoglobin method requires two reagents.

Procedure Limitations
Samples with Plasma Hemoglobin values greater than 100 mg/dL should be diluted with charcoal stripped human serum and re-assayed. Multiply results obtained by dilution factor.

Analytical Parameters	**Spec.**	**Titer plate**
Wavelength	650nm	650nm
Temperature	37.0°C	37°C
Pathlength	1 cm	0.212cm
Reaction Mode	Two point	Rate
Reaction Time	~3 minutes	~3 minutes
Reaction Volume (R1)	0.500 mL	150uL
Reaction Volume (R2)	0.200mL	60uL
Sample Volume	0.006mL	2uL
Total Volume	0.706 mL	212uL
Sample-to-reagent ratio	1:118	1:106

Assay Procedure
1. Plasma Free Hemoglobin reagents are ready to use.
2. Set the spectrophotometer temperature at 37°C, wavelength at 650nm and zero the instrument with a reference cuvette containing water.
3. Pipette 0.5 mL of R1 Reagent into each of four cuvettes marked: "Blk", "Test(UN)", "Calibrator", "Control".
4. Pipette 0.006 mL of Blk (water), Test sample, Calibrator, and Control into their respective cuvettes.
5. At timed intervals, add 0.200mL of R2 Reagent to each reaction cuvette. Mix each cuvette immediately by inversion.
6. Exactly **1 minute** after addition of R2 Reagent, read absorbance (A) of each cuvette marked Blk, Test sample, Calibrator, and Control at the set wavelength of 650nm against water as reference.

Calculations and Results

$$\text{UN (mg/dL)} = \frac{\text{(A) Sample} - \text{(A) Blank}}{\text{(A) Calibrator} - \text{(A) Blank}} \times \text{calibrator(mg/dL)}$$

Example
(A) Blank = 0.05
(A) Test = 0.25
(A) Calibrator = 0.50
Calibrator = 25 mg/dL

$$\text{Unknown PFH test values} = \frac{0.25 - 0.05}{0.50 - 0.05} \times 25 = 1.1 \text{mg/dL}$$

Reference Values (5)
None detected ≤ 5 mg/dL

The value given here is only to be used as a guideline. It is recommended that each laboratory establish the normal range for the sample source and geographical area in which it is located.

Interfering Substances (7)
The following substances have no significant effect on the accuracy of this Plasma Free Hemoglobin procedure at the concentrations stated.
- Bilirubin ≤ 20.0 mg/dL
- Ascorbic acid ≤ 2.00 mg/dL

Other factors such as hemolysis during and after venipuncture, lipemic plasma, turbidity, and methemalbuminemia may cause falsely elevated values in the Plasma Hemoglobin assay.

Method Performance Characteristics
Linearity: This procedure is linear over a range of 0-100 mg/dL (0-5.600 mmol/L).

Limit of Detection: 0.120mg/dL (as determined on a Beckman AU 480)
Limit of Quantification: 1.20mg/dL (as determined on a Beckman AU 480)
Precision: Precision data was obtained using five levels of serum-based controls on a Beckman AU480.

The following results were observed:

Plasma Free Hemoglobin	**Within- Run Precision**		**Total Precision**	
Mean	**SD**	**CV**	**SD**	**CV**
mg/dL	**mg/dL**	**%**	**mg/dL**	**%**
3.9	0.15	3.98	0.17	4.92
26.4	0.90	3.42	1.16	4.51
54.4	1.19	2.81	1.53	3.71

Bibliography

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