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Product Information

Drabkin's Reagent

Catalog Number **D5941**
Store at Room Temperature

Product Description

Drabkin's Reagent is used for the quantitative, colorimetric determination of hemoglobin concentration in whole blood at 540 nm.

Classic techniques for determining blood hemoglobin were based on estimation of oxygen, carbon monoxide capacity, or iron content. These assays proved unreliable because of the heterogeneous nature of hemoglobin. A colorimetric cyanmethemoglobin method was proposed where total hemoglobin at alkaline pH is rapidly converted to the cyanoderivative.[1] The absorbance of the cyanoderivative is determined at 540 nm. The method was simplified by combining the separate reactants, alkaline ferricyanide and cyanide, into a single reagent.[2]

Drabkin's Solution reacts with all forms of hemoglobin except sulfhemoglobin, a pigment that normally occurs in only minute concentrations in blood. The broad absorption peak of cyanmethemoglobin permits its measurement using both wide and narrow bandwidth instruments (530–550 nm).

Sigma provides a stable, dry Drabkin's Reagent, which is combined with a surfactant to prepare Drabkin's Solution. The surfactant minimizes turbidity sometimes caused by the presence of erythrocyte stroma.

This procedure is based on the oxidation of hemoglobin and its derivatives (except sulfhemoglobin) to methemoglobin in the presence of alkaline potassium ferricyanide. Methemoglobin reacts with potassium cyanide to form cyanmethemoglobin, which has maximum absorption at 540 nm. The color intensity measured at 540 nm is proportional to the total hemoglobin concentration.

Component

Drabkin's Reagent Catalog Number D5941 Contains sodium bicarbonate, potassium ferricyanide, and potassium cyanide	6 vials

Reagents and Equipment Required but Not Provided

- Brij® L23 Solution, Catalog Number B4184, 30% (w/v) Brij L23 solution. If solution solidifies, warm to 37 °C to liquefy
- Appropriate hemoglobin for preparation of standard curve
- Spectrophotometer capable of measuring absorbance at 540 nm
- Cuvettes
- Test tubes
- Pipetting devices for the accurate delivery of volumes required for the assays

Precautions and Disclaimer

Drabkin's Reagent and Brij L23 solution are for R&D use only, not for *in vitro* diagnostic use, drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

To prepare the Drabkin's Solution, reconstitute one vial of the Drabkin's Reagent with 1,000 mL of water. Then add 0.5 mL of the 30% Brij L23 Solution, Catalog Number B4184, to the 1,000 mL of reconstituted Drabkin's Reagent. Mix well and filter if insoluble particles remain.

Storage/Stability

Store the Drabkin's Reagent at room temperature protected from light. The powdered Drabkin's Reagent is stable for at least 2 years.

The prepared Drabkin's Solution is stable for at least 6 months stored at room temperature protected from light (amber bottle).

Procedure

Capillary or venous blood may be used as samples. Specimens obtained by capillary puncture should be free flowing and pipetted immediately into Drabkin's Solution, rinsing the pipette 3–4 times in the reagent. Venipuncture specimens must be collected in tubes containing solid anticoagulants, such as oxalate, citrate, EDTA, or heparin. After thorough mixing with the anticoagulant, the blood can be frozen for as long as 2 years[3] or stored for at least a week at 30 °C.[4]

Substances that cause turbidity will influence the absorbance measurement of the cyanmethemoglobin. These include lipids,[4] abnormal plasma proteins,[5] or erythrocyte stroma.[6]

1. Set the spectrophotometer wavelength to 540 nm and the absorbance to zero using water as the reference.

2. Set up a series of labeled test tubes for Blank and Tests.

3. To all tubes, add 5.0 mL of the Drabkin's Solution.

4. To each tube labeled Test, add 20 µL of the whole blood sample, rinsing the pipette 3–4 times with reagent. Mix well and allow to stand for at least 15 minutes at room temperature (18–26 °C). Note: Samples with appreciable carboxy-hemoglobin content may require a longer reaction time under these reaction conditions. Alternatively, it has been suggested that in those cases, warming the reaction mixture at 56 °C for 3–5 minutes with gentle mixing will bring the reaction to completion.[7]

5. Read and record absorbance (A) of each Test versus the Blank as the reference at 540 nm in the same instrument used to prepare the calibration curve.

6. Determine the total hemoglobin concentration (mg/mL) of each Test directly from the calibration curve. Color is stable for several hours.

Calibration Curve

1. Prepare a Cyanmethemoglobin Standard Solution containing 180 mg/mL of appropriate hemoglobin prepared in Drabkin's Solution. Store tightly capped and refrigerated (2–8 °C) in the dark.

2. Prepare a Dilute Cyanmethemoglobin Standard Solution by adding 40 µL of the prepared Cyanmet-hemoglobin Standard Solution (step 1) to 10.0 mL of the Drabkin's Solution.

3. Prepare working standards by pipetting and mixing thoroughly the solutions indicated in Table 1.

Table 1.
Preparation of Working Standards

Std. Tube No.	Dilute Cyanmet-hemoglobin Standard (mL)	Drabkin's Solution (mL)	Cyanmet-hemoglobin Concentration (mg/mL)
1	0.0	3.0	Blank (0)
2	1.0	2.0	60
3	2.0	1.0	120
4	3.0	0.0	180

4. Read absorbance of Tubes 2–4 versus Tube 1 as the reference at 540 nm.

5. Record the absorbance values.

6. Plot a calibration curve of absorbance values versus the cyanmethemoglobin concentration (mg/mL). The curve is linear, passing through the origin.

References

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