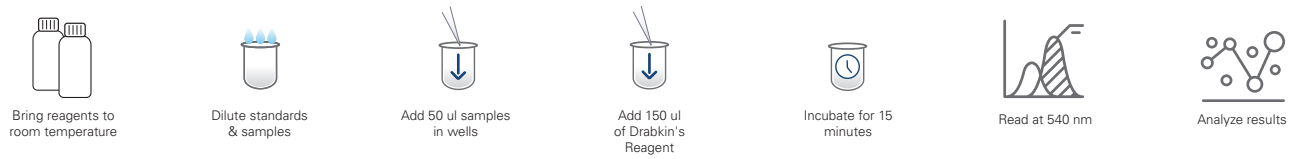
Exocell™

# DRABKIN'S MICROPLATE HEMOGLOBIN ASSAY




## DESCRIPTION

Drabkin's Microplate Hemoglobin Assay is for the determination of hemoglobin in blood samples, column fractions, and any sample with visible hemoglobin. It is for research purposes and is not intended for diagnostic use.
Description: The Drabkin's Microplate Hemoglobin Assay converts the variable hemoglobin forms in blood samples to cyanmethemoglobin which can be read spectrophotometrically at 540 nm. Compared to the standard cuvette-based assay, the microplate assay uses less sample and is amenable to high thruput. The dose response of the assay is linear from 0.5 mg/ml to 20 mg/ml.

Hemoglobin can exist in multiple forms depending on oxygen content, carbon monoxide content, and oxidative state of the iron (ferrous, ferric, and ferryl). Each form has a unique extinction coefficient and peak absorbance wavelength. The heterogeneous nature of hemoglobin can be overcome by conversion to cyanmethemoglobin. This is achieved by reaction of hemoglobin with alkaline ferricyanide and cyanide in a single reagent. The resulting cyanmethemoglobin has a peak absorbance at 540 nm and an extinction coefficient of 0.68 for a 1 mg/ml solution.

## KIT CONTENTS

- 1 x 96-well Assay Plate
- 1 x Hemoglobin Standard
- 2 x Drabkin's Reagent

**Other Materials required but not provided:**
- Microplate reader equipped to measure absorbance at 540 nm is required
- Adjustable pipettes and pipette tips
- Multi-channel pipettor(s) and tips for dispensing 150 µl volumes
- Tubes for preparing dilutions, i.e. microfuge tubes

## SPECIMEN COLLECTION AND STORAGE

**Blood:** Venipuncture samples should be collected in tubes containing solid anticoagulants. Samples can be stored frozen for several years.
**Samples:** Any sample, such as column fractions, that contains visible hemoglobin can be used in the assay.

## LIMITATIONS

- FOR RESEARCH USE ONLY, NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- It is the responsibility of the investigator to determine if the presence of experimental compounds or their metabolites in the sample that may affect the assay results.
- Any compound that absorbs light at 540 nm will affect the assay results.
- Compounds that cause turbidity, such as lipids, abnormal plasma proteins or erythrocyte stroma, will affect the assay results. Centrifugation to clarify sample may be necessary.
- Do not mix or substitute reagents with those from other kits or sources.
- Do not use the kit beyond the expiration date on the kit label.

## ASSAY PROCEDURE

This procedure describes the control, standard and sample dilutions, and their addition to the plate. Standard and sample

dilutions are assayed in duplicate wells which allows analysis of up to 40 samples.
Allow reagents and samples to come to room temperature before running the assay. (Note: the assay performs better when room temperature is between 20oC and 25oC.)
Allow frozen samples to thaw at room temperature, and gently mix to assure homogeneity. Leave the samples undisturbed for 30-60 minutes to allow particulates to settle out.

**Standard Dilutions**
This procedure describes the serial dilution of hemoglobin standard. There is enough standard to produce 4 curves. Unused wells of the plate and reagents can be saved for future experiments.
1. Label the tubes numbers 1-7.
2. Add 120 µl of Drabkin's Reagent per tube.
3. Transfer 120 µl of Hemoglobin Standard Stock to tube 1; this is a 1:2 dilution of the standard.
4. Mix contents by aspirating and expelling the fluids 5 times.
5. Transfer 120 µl of solution from tube 1 to tube 2.
6. Mix as before.
7. Continue this procedure through tube number 7.
8. Tubes 1-7 now contain dilutions representing 20, 10, 5, 2.5, 1.25, 0.625, 0.3125 mg/ml hemoglobin.

**Preparation of Sample Dilutions**
Samples must be diluted to fall into the range of the assay.
1. A starting dilution of 1:15 in Drabkin's Reagent for blood specimens is suggested, though for initial studies; it is wise to complete the analysis at more than one dilution.
2. A starting dilution of 1:5 in Drabkin's Reagent for column fractions is suggested, but it is wise to complete the analysis at more than one dilution.
3. It is recommended that sample dilutions be performed in tubes; dilution in the plate is not recommended.

**Preparation of Drabkin's Reagent**
The Drabkin's Reagent is provided as a ready to use solution.

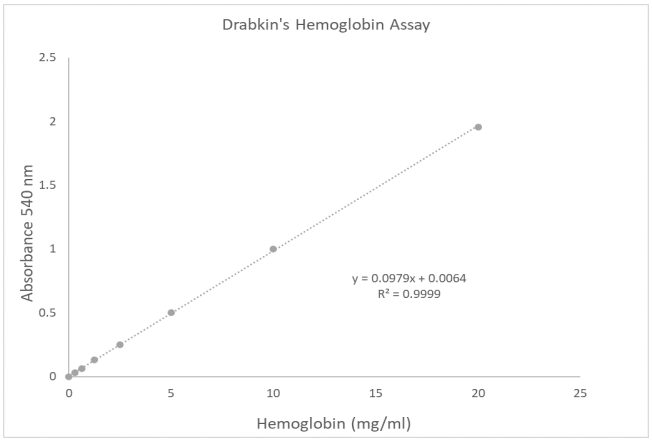
**Addition of Controls, Hemoglobin Standard Dilutions and Samples to the plate:** The plate design described here includes a negative control (Assay Diluent). This is placed in wells A1, and A2. All other wells receive either diluted standard or diluted sample
1. Add 50 µl Drabkin's from the stock bottle to wells A1 and A2. This is the negative control and will be used to standardize or "blank" the microplate reader.
2. With a fresh tip, transfer 50 µl aliquots of Hemoglobin Standard Dilution Tube 1 (1:2 dilution of stock) to wells B1 and B2.
3. With a fresh tip, transfer 50 µl aliquots Hemoglobin Standard Dilution Tube 2 to wells C1 and C2.
4. Continue transferring diluted standard to the plate in this fashion, i.e. in order through H1 and H2, taking care to use a fresh tip for each new dilution.
5. Using a new tip, add 50 µl aliquots of Diluted Sample to wells A3 and A4.
6. Continue adding diluted samples to the plate, taking care to change the tip for each one.
7. The plate now contains controls, standard dilutions, and diluted experimental samples in duplicate.

**Addition of Drabkin's Reagent:**
1. Add 150 µl Drabkin's Reagent to each well of the plate. A multichannel pipettor is recommended.
2. Incubate the plate at room temperature for 15 minutes.

**Measure the OD at 540 nm:** Use a plate reader to determine and record the absorbance of all wells at 450 nm, blanked against well A1.

**Analysis:** Color development in the assay is proportional to the hemoglobin concentration of the diluted standard or sample. Computer-based curve-fitting software program for a linear model: fitting mean absorbance (y axis) against the protein concentration (x axis). The hemoglobin concentration of the samples can then be interpolated from the standard curve. Multiply the concentration by the dilution factor to determine the undilute sample concentration. Alternatively; prepare a spreadsheet entering appropriate data including standard dilution, concentration, sample dilution and absorbance data. Determine the mean for replicate wells.

**Prepare a linear plot of the standard curve:** Place the [Hemoglobin] on the x-axis and the mean absorbance on the y-axis. The data that fall into the dose-response curve constitute the usable portion of the assay.




Subject these data to linear analysis to yield a mathematical model, of the form:

A450 = m x [Hemoglobin] + b

which rearranges to:

[Hemoglobin] = (A450 – b) / m

Multiply by the dilution factor of the sample to determine the concentration of undilute sample.

**Quality Control:**
**Record Keeping:** It is good laboratory practice to record the lot numbers and dates of the kit components and reagents for each assay.
**Sample Handling:** The samples should be secured, processed and stored as discussed above.
Dilute Standard and Samples carefully. For each standard and sample, a fresh tip should be used.
**Template:** Record the position of each standard or sample on a microplate template.

## TROUBLE SHOOTING

- Color in sample well(s) is darker or lighter than highest or lowest concentrations of the standard curve. Change sample dilution protocol appropriately.
- Poor agreement between duplicate wells: This is almost always due to pipetting error. Repeat the assay.

## REFERENCES

1. Meng, F. and Alayash, A.I. (2017) Anal Biochem. 521: 11-19.
2. Arnaud, F. et al. (2017) Artificial Cells, Nanomedicine, and Biotechnology 45:58-62.
3. International Committee for Standardization in Haematology (1967) Brit. J. Haemat. 13 (suppl.): 71

Drabkin's Microplate Hemoglobin Assay, v 1.0, June 10, 2022



## PRODUCT INFORMATION

| Cat. # | Description |
|---|---|
| 1044 | Drabkin's Microplate Hemoglobin Assay |

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