

Pyridine hemochromogen

HEME QUANTITATIONS FROM PYRIDINE HEMOCHROMOGEN SPECTRA

Hemochromogen formation

- Prepare a pyridine reagent mix by adding 3mL 1M NaOH and 6mL pyridine (use glass pipet) to 19mL H₂O in a glass container (28mL total volume).
- Put 1000μL of the pyridine reagent in a cuvet
- Add 35μL sample containing ≥5μM hemin.
- Add 18μL aqueous 15mM K₃Fe(CN)₆, mix well.
- Record the oxidized spectrum at 500-650nm. Repeat until stable.
- Add 2-5mg powdered Na₂S₂O₄ (sodium hydrosulfite, sodium dithionite).
- Mix well under the surface of the liquid mixture (minimize mixing with air).
- Record the reduced spectrum and repeat until stable.

Calculations

- For total heme (mM) subtract the absorbance readings at 540, 556 and 575nm in the oxidized spectrum from the corresponding readings in the reduced spectrum to get ΔA540, ΔA556 and ΔA575. (For example, ΔA540 = A540 Na₂S₂O₄reduced - A540 K₃Fe(CN)₆oxidized, etc.)
- Calculate [hemin, mM] in the mixture using the extinction coefficients:
 - 20.7/mM for ΔA540 ((ΔA556-ΔA540)/20.7)
 - 32.4/mM for ΔA575 ((ΔA556-ΔA575)/32.4)
- Factor in the dilution of the sample (30x or (1000+35+18)/35) and average the two results.
- For heme a, b, or c, multiply the difference of reduced minus oxidized absorbance at the five different wavelengths below and add the resulting five multiplication products.

Reference Information

From: Berry, E.A. and Trumpower, B.L. 1987. Simultaneous determination of hemes a, b, and c from pyridine hemochrome spectra. *Analytical Biochemistry*. **161**: 1-15.

Table 4

Inverse matrix of extinction coefficients of pyridine hemochromes for calculating concentration (mM) from reduced minus oxidized absorbance at five different wavelengths.

Wavelength (nm):	540	549	558	588	620
Heme c:	-0.02778	0.04757	-0.01906	0.00084	-0.00157
Heme b:	-0.02943	-0.00088	0.04037	-0.00267	-0.00738
Heme a:	-0.02687	0.00456	0.00699	0.04353	-0.02820

Total heme may also be obtained by adding the results for hemes a, b and c for a sample. Authentic heme solutions with known concentrations may be used to check the method and also to help check that the calculations were performed correctly to obtain expected results.

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