

# 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 H<sub>2</sub>O 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 K<sub>3</sub>Fe(CN)<sub>6</sub>, mix well.
- Record the oxidized spectrum at 500-650nm. Repeat until stable.
- Add 2-5mg powdered Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> (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 ΔA<sub>540</sub>, ΔA<sub>556</sub> and ΔA<sub>575</sub>. (For example, ΔA<sub>540</sub> = A<sub>540</sub> Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> reduced - A<sub>540</sub> K<sub>3</sub>Fe(CN)<sub>6</sub> oxidized, etc.)
- Calculate [hemin, mM] in the mixture using the extinction coefficients:
  - 20.7/mM for ΔA<sub>540</sub> ((ΔA<sub>556</sub>-ΔA<sub>540</sub>)/20.7)
  - 32.4/mM for ΔA<sub>575</sub> ((ΔA<sub>556</sub>-ΔA<sub>575</sub>)/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 Trumppower, 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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