FICIN ACTIVITY DETERMINATION
Updated September 2013

I. Personal Protective Equipment:
- A. Lab coat
- B. Safety glasses/goggles
- C. Latex gloves

II. Reagents:
- A. Denatured hemoglobin powder
  1. Add 20 g denatured hemoglobin/liter assay buffer
- B. Stock phosphate buffer, pH 6 0.5 M
  1. Add 10.54 g Potassium Phosphate (K_2HPO_4) (dibasic)/liter
  2. Add 59.66 g Potassium Phosphate (KH_2PO_4) (monobasic)/liter
- C. Working phosphate buffer, pH 6 0.1 M
  1. Add 200 ml stock buffer/liter
  2. Add 6.1 g cysteine hydrochloride ($C_3H_7NO_2S \cdot HCl$)/liter
- D. 30% solution of trichloroacetic acid (TCA) ($C_2HCl_3O_2$) (w/v)
- E. Enzyme solution
  1. Dissolve enzyme in working buffer to give absorbance reading of 0.1 to 0.7 when substrate (denatured hemoglobin) is added. Enzyme concentrations need to be in the range of 0.1 to 1.0 mg/ml. (Run duplicate tubes per enzyme concentration with duplicate blanks containing hemoglobin substrate but no enzyme.)

III. Assay:
- A. Add 5 ml of hemoglobin substrate to each tube
- B. Place in water bath at 39°C for 15 min
- C. Add 1 ml of enzyme and buffer solution to each tube (add only 1 ml of buffer to blanks)
- D. Stopper, swirl and incubate at 39°C for exactly 30 min
- E. Add 2 ml of 30% TCA to each tube, VORTEX to stop the reaction and precipitate protein
- F. Centrifuge at 4800 x g for 15 min
- G. Transfer supernatant to Quartz curettes with Pasteur pipettes and read at 278 nm on a spectrophotometer
- H. Adjust spec. to zero with blank before reading test samples.

IV. Calculation:

Calculate activity in Spectrophotometric Hemoglobin Units (SHU). SHU/g is the amount of enzyme that liberates 1 µmole of tyrosine ($C_9H_{11}NO_3$) per minute under the assay condition.

$$SHU/g = (A)(V)/(E)(T)(W)$$

Where:

A = Adjusted absorbance reading of the assay
V = Total assay volume (8ml)
E = Micromolar extinction coefficient for tyrosine (1.38)
T = Incubation time of assay (30min.)
W = Weight in grams of enzyme added to the assay