PART 2. XANTHINE OXIDASE ASSAY

This week's lab will familiarize you with the assay for measurement of xanthine oxidase activity in your isolated enzyme prep.

Resuspend the protein pellet in a total of 3.5 ml of resuspension buffer (50 mM phosphate buffer, 1.0 mM Na₂ EDTA, pH 8.0). Be careful not to make the solution too dilute (no measurable activity) or too concentrated (activity too high to measure). Store the enzyme on ice for future use.

The standard assay measures the change in absorbance (ABS) of the assay medium containing substrate, indicator dyes and enzyme. The rate of color change (ABS) is proportional to xanthine oxidase activity.

**Standard Assay**

**Buffer**
- 5.0 ml 50 mM Tris, pH 8.0

**Substrate**
- 0.1 ml 5 mM Xanthine or Hypoxanthine

**Dyes**
- 0.5 ml 2,6-Dichlorophenol-indophenol (DPIP)
- 0.1 ml Phenazine Methosulfate (PMS)

**Enzyme**
- 0.1 ml dilute enzyme

Prepare several assay test tubes, leaving out the enzyme. Measure the absorbance of one assay tube at 600 nm. To start the assay, add the enzyme, quickly vortex the tube and place it in the spectrophotometer. Take your initial reading (t₀) and then take readings at 10 sec intervals. The observed decrease in ABS over time is due to reduction of DPIP. Repeat the assay, adjusting the protein concentration of your enzyme prep until a rate of approximately 0.100 to 0.150 ABS/min is obtained. Be careful to initiate the reaction by adding the enzyme last and mixing rapidly since the reaction can proceed quickly. You should determine the reaction rate for your enzyme prep with 5 replicate assays for each substrate. Results should be expressed as mean specific activity and standard deviation of the mean (x ± sd, n=5). This means you must determine the protein concentration of your enzyme prep.

Individual rate curves should be constructed for each assay and rates (dA/dt) calculated from the initial linear portion of the curve.
The PMS couples the electron transfer from substrate to DPIP, which is a leuco dye, changing from blue (oxidized form) to colorless (reduced form) on transfer of reducing equivalents from PMS.