

## Starch Swelling Assay

### Procedure

- 1) If the starchy pellet from a Wallace extraction is to be used, wash the pellet 3 times with deionized water and dry completely in the speed-vac. (It is convenient to weigh the tube before weighing the flour so the mass of the remaining starch can be easily determined.)
- 2) Prepare a 1:4 [w:v] slurry of dried starch in deionized water with a small amount of bromophenol blue to provide contrast. Allow the samples to swell at least 1 h at room temperature.
- 3) Vortex the slurry to suspend starch granules evenly and quickly draw a sample into a hematocrit tube. The capillary and tube may need to be tilted below horizontal to fill well. Stop the flow by placing your finger over the end of the capillary tube. Seal with Critoseal putty. Prepare duplicate tubes for each sample to increase reliability of the assay.
- 4) Place the sealed tube in the holders of the Critoseal tray and allow the samples to settle overnight.
- 5) Place the tubes on the bed of a scanner and scan to acquire an image of the tubes. Save as a grayscale tiff file.
- 6) Use NIH Image (jImage, installed on Larkins4) to measure the length of the entire sample and then the length of the column of starch. Do not include the sealing putty in the measurement! Calculate the sedimentation coefficient by dividing the length of the starch column by the length of the starch plus liquid.

### Solutions and Supplies

Deionized water plus 0.05% Bromophenol Blue	Hematocrit tubes (VWR 15401-650) Sealing Putty (BD Labware VT1050 or Oxford 8889-215003)
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