## Collecting phloem exudations from cut stems into EDTA solutions

Based on

**Srivastava AC, Ganesan S, Ismail IO, Ayre BG** (2008) Functional characterization of the Arabidopsis AtSUC2 Sucrose/H+ symporter by tissue-specific complementation reveals an essential role in phloem loading but not in long-distance transport. Plant Physiol. **148:** 200-211

**van Bel AJE, Hess PH** (2008) Hexoses as phloem transport sugars: the end of a dogma? J. Exp. Bot. **59:** 261-272

**King RW, Zeevaart JA** (1974) Enhancement of phloem exudation from cut petioles by chelating-agents. Plant Physiol **53:** 96-103 (original description)

Required Items:

Plant material

5 mM EDTA solution (pH 6, autoclaved)

Clean plastic ware: 24-well microtitre plates or Coulter cuvettes; 3 for each sample

Ice and liquid nitrogen, chloroform, labeled microcentrifuge tubes

**Notes**: The EDTA-exudation method is used to collect phloem sap from cut petioles or from the cut stems of Arabidopsis rosettes. EDTA chelates the Ca2+ ions required to plug damaged sieve tube elements. EDTA is toxic to plant materials, so using dilute solutions (5mM, pH 6.0) and minimizing uptake via the xylem is important. Also, enzymes from cut plant material (*e.g.* invertases) can degrade compounds in the phloem sap and it is important to immediately chill/freeze exudation solutions after collection and inactivate enzymes with a chloroform extraction.

1. Grow plants under appropriate conditions for the required length of time. Individual leaves can be used from large plants or entire rosettes can be used from large or smaller plants. The size and type of chamber is used for exudates collection is based on the nature of the plant material. Label the plants with 14CO2 if required.
2. Consider and record the time of day that the experiment is started. Estimate the time required to process each plant (1 or 2 min?) and stagger processing so that the time points described below can be maintained. Organizing your work area is critical and you should arrange to have someone help you: e.g., working as an assembly line, one person makes the first cut, cleans and weighs the tissue (take the balance to your work area) and records the weight; the second person makes the second cut and arranges the material in the pre-prepared exudation chamber.
3. For whole rosettes, use small sharp scissors to cut below the hypocotyls in the uppermost part of the root.
4. Working quickly but carefully, remove any soil clinging to the plant and record the rosette fresh weight.
5. Submerge the cut end of the stem in 5 mM EDTA and keeping the stem submerged, use a sharp razor to cleanly slice the stem in the hypocotyl region (~>5 mm up from the first cut; a double sided razor, snapped in half, works very well.)
6. Arrange the prepared rosette so the cut stem in submerged in ~2 mm of 5 mM EDTA solution: 500 µL in the wells of a 24-well microtitre plate or 1 mL in a Coulter counter cuvette works well. The goal is to keep the cut end submerged, but to limit EDTA exposure to the rest of the plant – test for the best volume and vessel before you start. Optional: This solution can/should contain 10 µM lactose as an internal standard.
7. Cap or cover the wells/cuvettes to maintain near 100% humidity and minimize the amount of EDTA solution drawn into the leaves by transpiration and xylem transport.
8. Collect exudates for 20 m, then transfer the plant to a 2nd microtitre plate well or cuvette submerging only the cut stem as described above. **Immediately** collect the first exudate solution into a labeled microcentrifuge tube and freeze in liquid nitrogen: this is critical to minimize degradation by enzymes from cut cells. These exudates will be analyzed later, but are nonetheless considered washes since they will have the contents of the cut cells.
9. Repeat step 8 after 60 minutes (80 minute time point), and repeat again after another 60 minutes (140 minute time point), collecting the exudation solution and freezing immediately after removing the plant material. The results of these two time points will be reported and should be consistent to show that the exudation is proceeding properly.
10. Add 500 µL chloroform the frozen exudates and thaw while mixing to destroy enzymes.
11. Centrifuge briefly to separate the phases and recover the aqueious phase to fresh microcentrifuge tube. Retain the chloroform phase if required.
12. The exudates can be stored at -80°C or processed further for analysis (concentrated by lyophilization; passed through ion exchange columns to isolate the neutral fraction; used in Dionex HPAEC PAD; enzyme assays; derivitization and metabolomics, etc.). Exuded compounds are expressed as nmoles (mg fwt)-1 hour-1.