Apoplastic fluid sampling (centrifugation)

**Materials**

- Razor blade
- 10 mL syringes (with the upper part cut out so they can fit in a centrifuge tube)
- Micropipettes, Eppendorf tubes
- Centrifuge and plastic centrifuge tubes (50 mL)
- Parafilm
- UV (quartz) cuvettes
- Spectrophotometer

**Sampling Procedure**

- Sample the whole leaves with the petioles in the growth chamber, and place them immediately in a glass container with Type I ultrapure water
- Sampling time should be 3-4 h after the light onset in the growth chamber
- Cut out the petiole approximately 0.2-0.4 cm from the leaf base
- Blot the leaf dry
- Roll up the leaf and place it in a 10 mL plastic syringe with the petiole side at the bottom end of the syringe
- Centrifuge at 2.500 g for 15 min at 4 ºC and discard the fluid. This is to remove xylem sap from the main vein
- Centrifuge at 4.000 g for 15 min at 4 C and collect the apoplastic fluid
- Place the apoplastic fluid on ice and analyze contamination immediately

**Notes:**

- Leaves should fit tightly in the syringe, if they are loose they will collapse in the bottom and the volume extracted will be absorbed into the tissue.
- Big leaves will collapse in the bottom and frequently break the syringe. To avoid that and to fit the leaf in the centrifuge tube, the upper part of the leaf can be cut off
- Place the petiole end as close to the syringe hole as possible

Assesment of cytosolic contamination by mdh activity

- Place all the chemicals and samples in an ice bucket
- Add to a UV (quartz) cuvette the following reactants in the order written in table

<table>
<thead>
<tr>
<th>Sample</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vol in µL</td>
<td>Vol in µL</td>
</tr>
<tr>
<td>water</td>
<td>680</td>
</tr>
<tr>
<td>Tris 0.465 mM pH 9.5</td>
<td>150</td>
</tr>
<tr>
<td>Oxalacetate 4mM</td>
<td>150</td>
</tr>
<tr>
<td>NADH 10mM</td>
<td>15</td>
</tr>
<tr>
<td>Apoplastic fluid</td>
<td>5</td>
</tr>
</tbody>
</table>

- Mix well by inverting 3 times the quartz cuvette, using Parafilm to cover the top
- Follow the decrease in Absorbance at 340 nm during 1 min
- Use the extinction coefficient ε = 6.3 M⁻¹ cm⁻¹ and the Beer-Lambert law to calculate activity. It is desirable to print the graph to assess that no bubbles or problems affected the measurement. Calculate the slope taking into account the whole time and not just the initial and final time points
- Contamination is given as a percentage of total leaf mdh activity, considering the density of the apoplastic fluid is 1

**Notes:**

- Contamination should be measured in fresh samples, because mdh activity decreases after
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Notes:

- Contamination should be measured in fresh samples, because mdh activity decreases after freezing
- NADH and OAA solutions can be stored at -20 ºC and Tris at 4 ºC (but re-check pH if buffer has been prepared for longer than a week).