## Sample Preparation

### 1 Buffer Preparation
- Take the buffer bottle out of the fridge. Check the buffer quality. It must be particle free and completely transparent.
- Prepare a 50 ml buffer aliquot
- Put the buffer bottle back into the fridge.
- Let the aliquot equilibrate to room temperature

**TIP:** The Pollen Analysis Instructions on www.amphasys.com/downloads contains the recommended buffers

**TIP:** For hydrophobic pollen, add Tween20 (final concentration 0.05 %) to the buffer aliquot to facilitate suspension

### 2 Pollen Collection

<table>
<thead>
<tr>
<th>Type</th>
<th>Quantity/Type</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PURE POLLEN</strong></td>
<td>Add the quantity of pollen for one measurement into a 1.5 ml Eppendorf tube. Recommended quantity:</td>
</tr>
<tr>
<td><strong>ANTHERS</strong></td>
<td>Add anthers into a 1.5 ml Eppendorf tube. Number of anthers: Wheat 3, Pepper 3, Potato 1, Brassicas 6, Cucumber 3, Melon 3, Watermelon 3</td>
</tr>
<tr>
<td><strong>WHOLE FLOWERS</strong></td>
<td>Add flowers into a 1.5 ml Eppendorf tube. Recommended quantities: Fennel: 1 umbellet, Carrot: 1 umbellet</td>
</tr>
</tbody>
</table>

**NOTE:** High cell concentration lead to a higher risk of chip clogging and inaccuracy of the data.

### 3 Rehydration (if required)
- If the sample material is dehydrated, rehydrate for 30 min. Place the open Eppendorf tube with the sample in a rehydration box and close the box.

**TIP:** The Pollen Rehydration Quick Guide shows how to determine the optimum rehydration time

### 4 Buffer

**TIP:** For anthers: If pollen cannot be released from the anther by shaking, slice them with a scalpel before adding the measurement buffer or squeeze them with tweezers after adding the buffer (step 5)
- Add 1 ml measurement buffer

### 5 Extraction and Suspension
- Suspend the pollen by finger flicking or moving the tube over a tube rack (see image)

**TIP:** For anthers: In case the pollen cannot be released from anthers by shaking, use tweezers to squeeze the anthers

### 6 Filtration
- Use the recommended filter type (see Pollen Analysis Instructions on www.amphasys.com/downloads). Filters must be clean and dry.

### 7 Dilution
- F and D chip: Add 1 ml measurement buffer
- E and G chip: Add 2 ml measurement buffer

### 8 Equilibration
- Let the pollen equilibrate for 2 – 3 minutes in the buffer

**Exceptions:** Sensitive pollen, such as wheat, brassica, artichoke. These samples must be measured immediately.

**TIP:** The stability of the cells in the buffer can be tested using the experiments suggested in the Stability of Pollen Cells Quick Guide

### 9 Mixing
- Slowly invert the FACS tube to distribute sedimented cells and immediately start measuring

**TIP:** Seal the FACS tube with Parafilm before inverting