



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
- | PURE POLLEN | ANTHERS | WHOLE FLOWERS |
|---|---|--|
| <ul style="list-style-type: none"> • Add the quantity of pollen for one measurement into an 1.5 ml Eppendorf tube • Recommended quantity: | <ul style="list-style-type: none"> • Add anthers into an 1.5 ml Eppendorf tube • Number of anthers:
Wheat 3, Pepper 3, Potato 1, Brassicas 6, Cucumber 3, Melon 3, Watermelon 3 | <ul style="list-style-type: none"> • Add flowers into an 1.5 ml Eppendorf tube • Recommended quantities:
Fennel: 1 umbellet
Carrot: 1 umbellet |



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

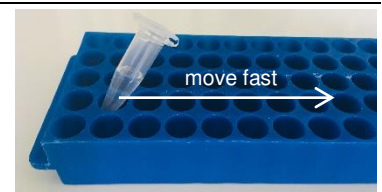
- 3 Rehydration (if required)
- If the sample material is dehydrated, rehydrate for 30 min. Place the open Eppendorf tube with the sample in a rehydration box and close the box.

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



- 4 Buffer
- Add 1 ml measurement 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)

- 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 chip: Add 1 ml measurement buffer
 - D chip: Add 1 ml measurement buffer
 - E 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