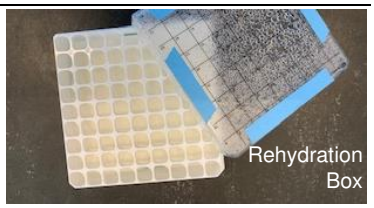
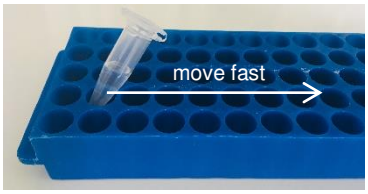
QUICK GUIDE: SAMPLE PREPARATION FOR POLLEN ANALYSIS

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** • Add the quantity of pollen for one measurement into a 1.5 ml Eppendorf tube • Recommended quantity:  **ANTHERS** • 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 **WHOLE FLOWERS** • Add flowers into a 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	***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*