QUICK GUIDE: OPTIMIZING POLLEN REHYDRATION

Introduction

The moisture content of pollen has an influence on their electrical properties. For long term pollen storage, cells are usually dehydrated. Dehydrated pollen cells can show similar impedance characteristics to nonviable cells. Therefore, it is important to rehydrate previously dehydrated cells in order to obtain an accurate determination of cell viability using the Ampha Z32 Impedance Flow Cytometer.

The following experiment can be used to determine the optimum sample rehydration time. Three dehydrated pollen samples will be rehydrated for five different durations and the viability will be measured for each. The rehydration duration resulting in the maximum measured viability is recommended.

Materials

- Rehydration box (Amphasys Starter Kit)
- 3 pollen samples large enough for 5 measurements each
- Measurement buffer, chips and filters
- Sample preparation materials
- Measurement template

Aliquotation Scheme

Sample	Rehydration time (min)				
A	0	15	30	45	60
B	0	15	30	45	60
C	0	15	30	45	60

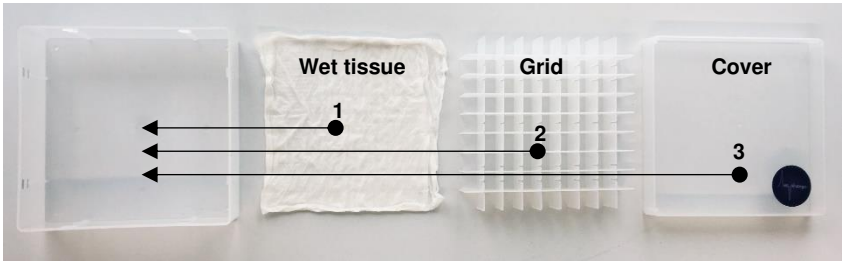
Protocol for the optimization of the rehydration time

Note: This protocol only applies to dehydrated pollen samples

1 Prepare Rehydration Box

- Open the rehydration box and remove the plastic grid.
- Place a wet paper tissue at the bottom of the rehydration box (step 1).
- Insert the plastic grid again (step 2) and cover the rehydration box (step 3).

Assembling the rehydration box



2 Prepare Instrument

- Prepare your Ampha Z32 instrumentation according to the instructions described in the Startup Procedure Quick Guide.

3 Homogenize Pollen

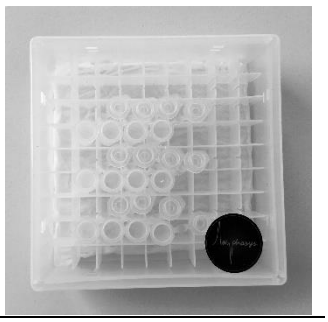
- Remove 3 dehydrated pollen samples from storage.
- Shake the tubes very well to homogeneously disperse the cells.

4 Aliquotation

- After homogenization, prepare 5 small aliquots of each of the 3 sample tubes in fresh 1.5 ml Eppendorf tubes. The amount of pollen in each tube should be just enough for one measurement.

  ***TIP:** Recommended pollen quantities can be found in the **Sample Preparation Quick Guide**.*
- Name the tubes with the numbers 1 to 5 and the sample name.
- Immediately put the tubes with numbers 2 to 5 into the rehydration box. Make sure the tubes are open.
- Cover the rehydration box.
  - Record the start time of the rehydration (e.g. 3.00 pm).

Rehydration box with aliquots



5	Measurement of Dehydrated Samples	• Immediately prepare the 3 sample with number '1' (not rehydrated) according to your sample preparation protocol and measured them directly. ***TIP:*** *In case you don't have a sample preparation protocol yet, have a look at the* ***Sample Preparation Quick Guide*** *or contact Amphasys Support.* ***TIP:*** *Make sure that you follow the same sample preparation protocol for all samples, i.e. use the same equilibration duration shown in the* ***Sample Preparation Quick Guide****.*
6	Measurement of Rehydrated Samples	• After 15 min rehydration, remove the aliquots with number '2', prepare the samples and measure them. • After 30 min rehydration, remove the aliquots with number '3', prepare the samples and measure them. • After 45 min rehydration, remove the aliquots with number '4', prepare the samples and measure them. • After 60 min rehydration, remove the aliquots with number '5', prepare the samples and measure them. ***TIP****: Make sure that you cover the rehydration box immediately after removing samples*
7	Data Analysis	• Determine the cell viability by gating. • Create a .csv report. ***TIP****: Tips and tricks for data analysis are shown in the* ***Data Analysis Quick Guide****.* • Plot the measured cell viability vs. rehydration time, e.g. using Microsoft Excel or R. • The time for which the maximum measured viability is obtained is the recommended rehydration time. ***TIP****: In case the measured viability does not significantly increase for longer rehydration times (e.g. 80 % for 15 min and 81 % for 30 min), the shorter rehydration time can be selected as the optimum.*