QUICK GUIDE 2: MEASUREMENT & TIPS

Measurement

1. Dispersion

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

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

  TIP: *If you intend to prepare larger series of samples, please check the stability of the cells in the buffer. Suggestions can be found in the Tips and Tricks section, page 2.*

  TIP: *Tapping at the sample container helps to prevent larger pollen from sedimenting during the measurement.*

2. Measurement

Recommended stop conditions

F-chip
10'000 cells and 3 minutes
for approximately 2 ml sample

D-chip
10'000 cells and 2 minutes
for approximately 2 ml sample

E-chip
10'000 cells and 45 seconds
for approximately 3 ml sample

- Put the sample under the sample holder
- Click **Start Measurement**. The measurement will start automatically after the loading process (9 – 12 seconds)
- Stop the measurement manually by clicking **Stop Measurement** or using stop conditions
- With default settings, a flush process is initiated automatically after stopping the measurement

  TIP: *In case you don't want to contaminate the remaining sample in the FACS tube with flushing water, remove the tube immediately from the sample holder after stopping the measurement and put an empty container under the sample holder.*

3. Quality Control

Concentration [cells/ml]	61'391
Accepted	10'239
Rejected [%]	3.7

Date/Time	2018-11-26 17:03
Duration	00:39

- Make sure the rejection and detection rates are below the following thresholds:
  - **Rejection rate < 10 %**
  - **Detection rate < 1000 cells / second**

    (= accepted cells / measurement duration in seconds)

TIP: *Samples with high cell concentrations have a higher risk of chip clogging. Dilute the samples if you have frequent cloggings.*

TIPS AND TRICKS

Sample collection considerations

Pollen viability on a single plant can vary significantly depending on where on the plant the sample is taken, the developmental stage of the flower, the time of the day and environmental influences.

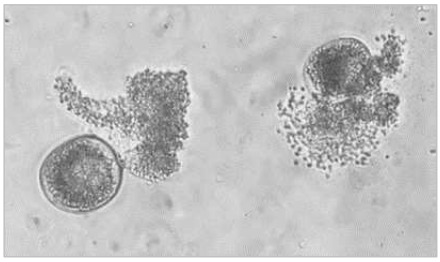
To compare pollen quality between plants, the following factors affecting pollen viability should be considered when collecting pollen:

- **Time of the day** – flower opening and pollen viability can vary strongly depending on the time
- **Variability within a plant** – compare pollen from flowers of a similar position on the plant and equal developmental stage
- **Environmental factors** – factors like the application of pesticides, humidity, temperature and light intensity can affect pollen viability

If you have a choice, work with pure pollen > isolated anthers > whole flowers/buds, as the amount of debris increases as the quality of the sample decreases from pure pollen to pollen extracted from whole flowers.

How to determine the stability of cells in buffer

Bursting Wheat Pollen



Methods to confirm that the pollen is stable in the chosen buffer are:

Microscopy

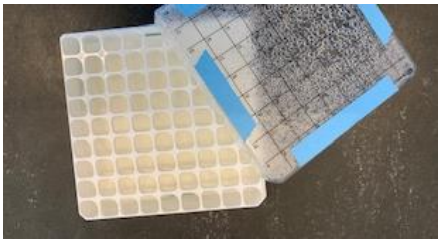
- Prepare a cell suspension with measurement buffer
- Pipet a drop on a glass slide and let the cells sediment
- Observe the cells under the microscope to see if any cells burst after 5, 10, 30 and 60 min

Ampha Z32

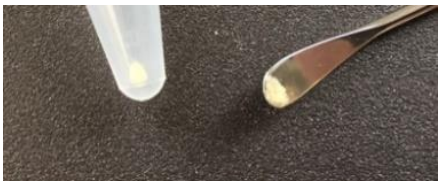
- Prepare a 10 ml sample and measure viability immediately after suspending the cells, and after 5, 10, 30 and 60 minutes
- If the viability decreases over time, the last point at which the viability is not decreased is the maximum recommended suspension time

How to determine the optimum rehydration time for dehydrated pollen

Rehydration Box



Amount of pollen for one measurement



- Prepare the Rehydration Box (see image) by placing a wet towel at the bottom of the box
- Disperse dehydrated pollen in the storage tube by shaking it well
- Prepare 7 small aliquots from the same sample in Eppendorf tubes. Take just enough pollen for one measurement (see image)
- Prepare one sample immediately and measure it
- Incubate the other 6 samples for 10, 20, 30, 40, 50 and 60 min in the humid air box before creating the cell suspensions and measuring
- Check for which rehydration time the measured viability does not increase any further. This is the recommended rehydration time.