QUICK GUIDE: POLLEN STORAGE



The following protocol outlines the planning and execution of an experiment to determine the pollen viability during pollen storage. This protocol can be used for the optimization of pollen storage conditions, e.g.

- Evaluation of different dehydration methods
- Evaluation of different storage conditions
- Determination of the maximum duration of pollen storage

Storage Protocol Evaluation

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| 1 Plan experiment | Define for how long you want to store the pollen (e.g. for 2 weeks, 1 year, 3 years) |
| | Define how often you want to check the pollen viability (e.g. daily, weekly, monthly) |
| | Define dehydration method(s) (e.g. silica gel, drying beads, heating furnace) |
| | Define storage method(s) (e.g80 °C, -20 °C, 4 °C, room temperature) |
| | Define suitable sample preparation protocol (see <u>Sample Preparation Quick Guide</u>) |
| | Extract pollen using your method of choice |
| 2 Pollen Extraction | Homogenize the extracted pollen by shaking the container well |
| 3 Aliquotation | Prepare aliquots of pure pollen |
| | Use the same sample containers that are used for pollen storage |
| | Prepare one aliquot per time point and condition, e.g. 26 for biweekly measurements during 1 year in a -80 °C freezer |
| | Use different materials, e.g. pollen from 3 different varieties |
| | Add more pollen into an aliquot than what is needed for one measurement (as much as possible) |
| 4 Measurement | Create a workspace for the measurement series and save the workspace |
| | • Take one aliquot and take a subsample to measure the pollen viability (t = 0 days, before dehydration) using the recommended sample preparation methodology. Don't forget to rehydrate the sample using a rehydration box, as it may have dehydrated during processing. |
| | Do not discard the aliquot. It will be used again in step 6 |
| 5 Dehydration | Dehydrate pollen sample aliquots using your method of choice |
| 6 Measurement | Take the aliquot used before and take another subsample to measure the pollen viability (t = 0 days, after dehydration) using the recommended sample preparation methodology. Don't forget to rehydrate the sample using a rehydration box. |
| 7 Storage | Store the pollen under the condition of choice |
| 8 Measurement | Remove one aliquot at each indicated time point (e.g. every 2 weeks) |
| | Homogenize the sample by shaking the tube well |
| | Remove a subsample from the tube. Just enough for one measurement. |
| | Measure the pollen viability using the recommended sample preparation methodology. Don't forget to rehydrate the sample using a rehydration box. |
| | Analyze the dataset, export the viability data and map it to the corresponding time points |
| 9 Data Analysis | Identify the best dehydration method, storage condition or storage duration |