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Preparing samples of Chlamydomonas cell paste for metal measurements (ICP-MS, ICP-AE)

1) count cells

2) collect 5 x 10^8 cells by centrifugation (JA17, 5min, 3500 rpm, 20 °C) using 50 mL falcon tubes, if your cell density is below 1 x 10^7 cells/mL repeatedly centrifuge cells into the same tube till you obtain the desired cell number.

> Note: see protocol below for preparing liquid samples if required.
>
> Comment: I prefer to always use the same number of cells for all experiments. However, that is not possible for all experiments and occasionally you have to use less cells. For ICP-MS the amount of cells between 2 and 5 x 10^8 cells gives reliable results.
>
> Important tips:
> a) do **not** process more than 7 samples at the same time (7 is the maximum number of tubes which fits in the JA17 rotor). Prolonged processing time tends to lyse cells and thus should be avoided. If you have more than 7 samples process them sequentially and not in parallel. This is especially true for a cell wall less strain!
> b) keep an eye on your pellets during all further steps: a white area within the green pellet is a sign for lysed cells, a yellowish ring surrounding the green pellet on the other hand suggests a contamination of the culture. **Be aware that your calculation in the end, e. g. metal atoms per cell, assumes full recovery of cells so you do not want to lyse nor lose cells!**
> c) do not exceed 4000 rpm otherwise the pellet gets too tight and is harder to resuspend.

3) Remove supernatant. Add about 5 ml of 1 mM EDTA pH8.0 to the tubes and resuspend cell pellet **gently** by swirling the liquid above the pellet. Do not vortex to resuspend cells! Do not resupend with a pipet!
Fill tube to the 50 mL mark with 1 mM EDTA pH8.0 and centrifuge at RT for 5 min at 3,500 rpm.

> Tip:
> Work fast. Do not keep the resuspended cells in the liquid too long, they might lyse. You can swirl the various samples together using the Styrofoam tray that the Falcon tubes come in.

4) Repeat step 3.

5) Remove supernatant. Resuspend pellet in 5 mL milliQ water and transfer cells to a 14 mL Sarstedt tube (rinsed in milliQ water beforehand, note: see preparing metal free tubes) using a plastic pipette. Wash previous tube with 5 mL milliQ water and combine with cell suspension in Sarstedt tube. Centrifuge at RT for 10 min and 10,000 rpm. Remove 9.5 mL of the supernatant.

Comment: I started to use 1 mM EDTA pH8.0 for this step instead of water to remove any traces of metals from the Sarstedt tubes. This means that I have to add an additional step, washing pellet with water, and therefore I centrifuge at 3,500 rpm at this step and 10,000 rpm at the next.

6) Do not resuspend cells! Carefully overlay pellet with 428 µL nitric acid (Commercial 70% stock in hood [Optima grade from Fisher, order number A467-500], final concentration of nitric acid is 30%). Take care to not disturb pellet: any cell material on the tube wall above the liquid phase will not be digested properly. Centrifuging to pellet this material will only make it worse.

Comment: I have recently added a short spin (5 min, setting 6) in the clinical centrifuge before adding the nitric acid. This moves the pellet nicely to the bottom of the tube and makes it easier to overlay it with acid.

Note: The final concentration of nitric acid has been reduced to 2% (ICP-MS at UCLA and CSULB) . Therefore, add 286 µL nitric acid instead of 428 µL and increase the volume of water added in step 8 to 9.2 mL.

7) Place tube(s) in rack and digest cell material in a water bath for 48 hours at 65°C. To avoid that the lids pop open place a glass plate on top of the tubes and hold it down using weights such as lead donuts

8) Add 9.1 mL of milliQ water to the digested material. Mix by inverting. Samples are now ready for measurement. Final nitric acid concentration is 3% (or 2%).

Preparing liquid samples for metal measurements (ICP-MS, ICP-AE)

If you want to measure the metal content of your initial medium simply take out 10 mL and add nitric acid (Optima grade) to the required final concentration. Be aware that your pipettes and tubes should be metal free (see protocol below).

If you want to measure medium after cell growth use metal free tubes for step 2 in above protocol. Transfer about 13 mL of the top supernatant by decanting carefully into a fresh, metal free 14 mL Sarstedt tube and spin them hard (5 min, 12,000 rpm) to remove all remaining cells. Transfer 10 mL into a fresh tube and add nitric acid (Optima grade) to the required final concentration. Be aware that your all pipettes and tubes should be metal free (see protocol below).

Preparing tubes for metal measurements (ICP-MS, ICP-AE)

1) Wash pipettes and tubes with detergent to remove all traces of grease and surface dust left over from production. It is even better to soak pipettes and tubes in detergent over night.

2) Rinse them thoroughly with de-ionized water

3) Submerge them in 10 % nitric acid (normal grade, not Optima grade!) and soak them for a week at 50 degree Celsius.

4) Rinse them thoroughly with milliQ water. Avoid unnecessary exposure of opened tubes to air as this might contaminate them.

Note:

- No leaching of metals from these tubes was detectable within a week.
- If necessary, pipettes and tips can be cleaned by pulling 10% nitric acid up and down followed by thorough rinse with milliQ water.