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Studying trace element nutrition without a clean room

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Trace elements are key to much of the chemistry that occurs in living cells. We have investigated trace element nutrition in the eukaryotic alga *Chlamydomonas reinhardtii*, and describe here common best-practices for studying trace element nutrition in the laboratory.

Jeanette M.Quinn, Sabeeha Merchant (2004). Copper-responsive gene expression during adaptation to copper deficiency. Methods in Enzymology.
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General best practices:

1 Wipe work area clean of dust, as dust is a common source of metal contamination.

2 Use fresh gloves when working with trace element grade chemical sources and media.

3 Use ultra-high purity chemical sources for buffers and growth media; specific batches with ultra-low trace element impurities can be acquired. It is advisable to request a Certification of Analysis to assess vendor batch-to-batch variation, and to note Lot Numbers used during experimentation where appropriate.

4 Acid wash vessels used for solutions and growth media (see Steps 10-19).

5 Keep chemical source bottles and stock solutions closed to air as much as possible. Limit the amount of time they are open. To maintain integrity of stock solutions, pour aliquots into temporary falcon tubes for regular access, rather than accessing the main stock container. This limits the amount of time stock solutions are exposed to air (which contains dust particles) and eliminates the need to insert vectors for contamination into the stock solutions.

6 Pouring is your friend. When possible, pour chemical powders and solutions instead of using spatulas or pipettes. Never use metal spatulas to scoop powders. When pipettes are to be used, it is best to use pipettes with minimal metal parts on the surface. We use Pipet-Lite™ XLS+ manual pipettes. Use fresh boxes of pipette tips which are open to air for minimal amounts of time, and individually wrapped single-use macropipettes for larger volumes. Filtered pipette tips can be used to further reduce contamination that can be introduced from pipettes.

7 When weighing chemicals, it is best to use a package of plastic weigh boats or weigh paper which have only been handled with fresh gloves. To prevent metal scales from contaminating weigh boats, place one weigh boat upside-down on the scale to act as a metal-free platform,

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then place the weigh boat destined for chemical powder atop the platform weigh boat.

8 When possible, dissolve solutions by shaking in solution manually. If needed, stir bars can be acid washed before use.

9 Prepare growth media within 1 week of use in acid washed glassware. Glass leaches metals, even after acid washing, so media should be made fresh if glassware is to be used.

Acid washing plastic bottles for stock solutions:

10 Prepare fresh 6N HCl to acid wash plastic graduated cylinders and plastic bottles for long-term stock solutions.

11 When possible, use new plastic bottles for stock solutions. Fill plastic bottle with 6N HCl. Incubate for >1 h right-side-up. Then flip upside-down and incubate >1 h additional to dissolve metals from the cap of the bottle as well.

12 Empty the HCl from the bottle. HCl solution can be reused ~3-4 times to dissolve metals, after which it should be neutralized and fresh 6N HCl remade.

13 Rinse the acid washed bottle 6 times with purified water (MilliQ): fill bottle completely with MilliQ, then pour out. Rinse cap as well.

> Take care not to let bottle or cap touch contaminated surfaces or remain open to air for extended amounts of time.

Acid washing glassware for experimentation:

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> Glass leaches metals, even after acid washing, so solutions in glassware should be made fresh within 1 week of use.

15 Fill glass vessel with 6 N HCl above the level that will be in contact with the solution or cell culture. Cover vessel with cling wrap, and incubate overnight.

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16 Empty the HCl from the vessel. HCl solution can be reused ~3-4 times to dissolve metals, after which it should be neutralized and fresh 6N HCl remade.

17 Rinse the acid washed vessel 6 times with MilliQ water: fill vessel with MilliQ, then pour out.

18 Keep vessel covered with cling wrap until the final cap is placed on the vessel for use or autoclaving.

Other notes:

19 It is OK to cover vessels for use within 1 week with aluminum foil. However, DO NOT cover vessels with aluminum foil *during* acid washing, as the acid will dissolve the aluminum.

20 Metal equipment should not / cannot be acid washed, as acid would simply continuously corrode the metal leading to contamination upon contact with trace element defined components.

21 To titrate pH of trace element solutions, similar precaution should be taken throughout:

Use designated high-purity sources of acid and base, solutions of which should be stored in clean plastic containers for use.

Do not place electrodes or pH strips directly into trace element defined solutions. Instead, either pipette solution onto pH strip, or pour into a small beaker for testing and then discard.

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