Fixation of isolated organelles for TEM and IEM

Contact EM office (room 457B, 48-6145 Ilkka) before starting new EM project or when collecting type of samples that have not been processed for you before!

TEM

- **TEM specimen fixative:** 1 % glutaraldehyde, 4 % formaldehyde in 0.1M phosphate buffer (pH 7.4).
- **Double concentrated fixative:** 2 % glutaraldehyde, 8 % formaldehyde in 0.1M phosphate buffer (pH 7.4).
    1. Add double concentrated fixative 1:1 to suspension.
    2. After 10 min spun organelles into pellet, remove fixative and add fresh fixative (normal strength).
    3. After 1 hour fixation centrifuge as above and leave the pellet in a fixative.
    4. Samples can be stored at +4^{o}C.
- **For shipping it is best to embed samples in agarose**
    1. Wash the pellet with PBS 3 x 10 min.
    2. Wash the pellet with water 2 x 10 min.
    3. Embed pellet in 2.5 % agarose.
        - Pipet a drop of agarose (2.5% in water, temperature around 37^{o}C) on top of pellet. Estimate the volume according to the size of your pellet.
        - Carefully dislodge the pellet so that it floats within agarose.
        - Cool agarose on ice
    4. Remove the agarose pellet e.g. by cutting the tip of the Eppendorf tube and trim agarose smaller. Organelle pellet should be surrounded by agarose.
    5. Store agarose pellet in the fixative.
    - **Bring / sent samples to EM lab in the fixative at RT. Make sure tubes fully filled with fixative, carefully closed, and lids are secured with parafilm.**
    - **Shipping address:**

      Biocenter Oulu / Electron Microscopy Laboratory
      Aapistie 5A
      90220 Oulu
      Finland

IEM

- **IEM specimen fixative**: 4 % paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) with 2.5 % sucrose.
- **Double concentrated IEM fixative:** 8 % paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) with 2.5 % sucrose.

1. Fix organelles in suspension by adding double concentrated fixative.
2. After 10 min, pellet organelles, resuspend in fresh fixative (4 % paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) with 2.5 % sucrose) and fix for 1-4 hrs (depending on the sensitivity of the antigen).
3. After fixation pellet organelles in Eppendorf tubes, remove the fixative and rinse the pellet twice with PBS.
4. Immerse pellet in a drop of 12 % gelatine in PBS (37^{o} C), loosen pellet very gently with a tip and incubate for 10 min at 37^{o} C.
5. Centrifuge at RT for 5 min (13 000 rpm).
6. Put tubes on the ice for 30 min,
7. Remove the gelatin pellet e.g. by cutting the tip of the Eppendorf tube and trim gelatin smaller. Organelle pellet should be surrounded by gelatin.
8. Transfer to 2.3 M sucrose in PBS at 4^{o}C and rotate for 4 hours at 4^{o}C.
9. Keep specimens in sucrose at 4^{o}C.

- **Bring / sent samples to EM lab in sucrose at RT. Make sure tubes fully filled with sucrose, carefully closed, and lids are secured with parafilm.**
- **Shipping address:**

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  Aapistie 5A
  90220 Oulu
  Finland

IMPORTANT!

- Every specimen tube must have a specimen number, date and type of the sample. Also mark whether sample is for IEM or TEM.
- With every set of specimens bring **completely filled** information sheet http://www.oulu.fi/sites/default/files/content/EM-core_order_form_2016.pdf
- When you bring the specimens to the EM lab: either leave them on the lab table (488B) with all information or to the cold room (480B) on 4th floor.

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