Fixation of cultured cells 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!

PROTOCOL FOR TEM

1. **TEM specimen fixative**: 1 % glutaraldehyde, 4 % formaldehyde in 0.1M phosphate buffer **or** 2 % glutaraldehyde in 0.1M phosphate buffer

- **Cells grown on plastic Petri dish** (enough cells for at least 2 mm^3 pellet):
    1. Remove cell culture media and add fixative (**at room temperature**).
        a. NOTE! If cytoskeletal structures are studied, fixation temperature can be even +37 ^oC.
    2. After 10 min detach cells carefully with a cell scraper and continue fixation for 1 hour maximum.
    3. Centrifuge cells to a pellet (6000 rpm, 2 min) and leave the pellet in Eppendorf tube in a fixative.
- **Cells grown in suspension culture**:
    1. Add double concentrated fixative 1:1 to suspension.
    2. After 10 min centrifuge (2000 rpm) remove the fixative and add fresh fixative (normal strength).
    3. After 1 hour fixation centrifuge as above and leave the pellet in a fixative. Cell pellet can be stored in a fixative at +4 ^oC.
- **Bring the cells to EM lab in a fixative**.

PROTOCOL FOR IEM

2. **IEM specimen fixative**: 4 % paraformaldehyde in 0.1 M phosphate buffer with 2.5 % sucrose

- Cells grown on a culture dish (enough cells for at least 2 mm^3 pellet):
    1. Remove cell culture media and using a Pasteur pipette put the fixative gently on cells.
    2. After 10 min, detach cells carefully with cell scraper and continue fixation for 1 hour (depending on the sensitivity of the antigen).
    3. After fixation transfer cells to the Eppendorf tubes and centrifuge (6000 rpm, 2 min).

a. **At this point you can bring samples to EM lab (contact lab personnel beforehand)**

4. Remove the fixative and rinse the pellet twice with PBS.
5. Put a drop of 12 % gelatine in PBS (37^{o} C) on the pellet, loosen pellet very gently with a tip and incubate for 10 min at 37^{o} C.
6. Centrifuge at RT for 5 min (13 000 rpm).
7. Put tubes on the ice for 30 min,
8. Remove the gelatin pellet e.g. by cutting the tip of the Eppendorf tube and cut the pellet to small pieces.
9. Transfer pieces to 2.3 M sucrose in PBS at 4^{o}C and rotate for 4 hours at 4^{o}C.
10. Keep specimens in sucrose at 4^{o}C and bring to EM lab.

- Frozen fixative and sucrose can be obtained from the EM lab (488B).

IMPORTANT!

- Every specimen tube must have a specimen number, date and type of the tissue. 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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