

Fixation of tissues 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!

1. **TEM specimen fixative:** 1 % glutaraldehyde, 4 % formaldehyde in 0.1M phosphate buffer (pH 7.4).
 - Fresh fixative is available in the cold room 480B. Bring your own tube!
 - **Fixation time:** 12 hours at room temperature, after which samples can be stored at +4° C in the fixative.
 - **When taking the sample it is IMPORTANT to**
 1. Cut the tissue quickly from the organ and put it immediately into the fixative.
 2. Further cut the tissue to 2 mm³ small pieces and transfer to the final fixative tube.
 3. Always take several samples (3-4), if possible.
 - a. **pieces from same sample should be put to same tube !!**
 4. The amount of fixative must be at least 10 times the volume of the tissue.
 5. Samples are stored and brought / sent to EM lab in a fixative.

2. **IEM specimen fixative:** 4 % paraformaldehyde in 0.1 M phosphate buffer with 2.5 % sucrose.
 - **Fixation time:** 2-6 hours or overnight at room temperature. Fixation time depends on the sensitivity of the antigen and needs to be determined experimentally.
 - **When taking the sample it is IMPORTANT to**
 1. Cut the tissue quickly from the organ and put it immediately into the fixative.
 2. Further cut the tissue to 2 mm³ small pieces and transfer to the final fixative tube.
 3. Always take several samples (3-4), if possible.
 4. The amount of fixative must be at least 10 times the volume of the tissue.
 - After fixation tissue pieces are transferred to 2.3 M sucrose in PBS, rotated for 4 hours at +4°C and stored at +4°C.
 - Samples are brought / sent to EM lab in sucrose (preferably at +4°C).

- 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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