

Sample Fixation and Agarose Embedding Protocols of Isolated Microorganism, Isolated Cell Organelle, and Cell Suspension for Conventional TEM Sample Preparation

Sample Fixation

1. No rinse required ahead of fixation.
2. Warm up a fixative provided by the Microscopy Core in water bath or incubator at 37 °C.
Note 1: The Core will provide 2.5% glutaraldehyde (GA) in 0.1M sodium cacodylate buffer for cell suspension.
Note 2: The Core will provide a special fixative for bacteria, yeast, or isolated pancreatic islet samples depending on your research purpose.
Note 3: The fixative should be warmed to avoid a temperature shock to sample.
3. Add the warm fixative of same volume as the sample medium. (volume of fixative/volume of medium = 1/1).
4. Gently mix and leave the fixed sample in culture bench or fume hood for 5 minutes.
5. Seal well with parafilm.
6. Store the fixed sealed sample at 4 °C until your TEM sample preparation service is scheduled.

Agarose Embedding

1. Making 3 - 4% low-melt agarose solution.
 - a. Add 0.3 - 0.4g low-melt agarose to a 20mL glass scintillation vial, then add 10mL distilled H₂O.
 - b. Microwave (~ 1,000 W) for 3 - 5 seconds with the glass vial cap slightly open.
 - c. Swirl the vial to completely dissolve the agarose.
 - d. Repeat b - c one more time if necessary.
 - e. Place aliquot of 3 - 4% agarose in a ~ 55 °C water bath and let cool for 5 minutes.
Note: Monitor solution during microwaving to prevent boiling over.
2. For microorganism and cell suspension centrifuge sample to form a pellet.
 - a. For cell suspension: 100 - 300 rpm for 10 minutes in maximum.
 - b. For bacteria: 1,000 - 2,000 rpm for 10 minutes in maximum.
 - c. For pancreatic islet: lightly tap Eppendorf tube on benchtop to condense islets in bottom of tube. If necessary, centrifuge at < 100 rpm.

- d. Remove as much supernatant as possible without disturbing pellet.

Note: Higher speed and longer time make sample elongated and subcellular structures dislocated.

3. Warm closed Eppendorf tube with the fixed sample at ~ 55 °C water bath for 5 minutes.
4. Using a pre-warmed pipette, add 100 - 150 µL of pre-warmed (~ 55 °C) liquid 3 – 4 % agarose to the warmed pellet sample.
5. For microorganism and cell suspension, resuspend the pellet in the agarose solution by gently mixing using pre-warmed pipet or fine tip spatula.

Note 1: Agarose should be warm to the touch but not too hot to handle.

Note 2: Care should be taken to not introduce air bubbles into the solution during the agarose addition and mixing steps.

6. Immediately place closed Eppendorf tube back in ~ 55 °C water bath for 3 minutes.
7. Take Eppendorf tube out of water bath and gently tap on benchtop to lightly mix the solution and remove air bubbles. Set back in ~ 55 °C water bath for 2 minutes.
8. Place agarose embedded sample at 4°C for 5 - 10 minutes to solidify.
9. Once solidified, separate the agarose button from the Eppendorf tube by gently sliding the pointed end of a spatula in between the agarose and the tube.
10. Place agarose button into appropriate fixative and store at 4 °C until submission to the Core for processing.