

Laboratory for Advanced Electron and Light Optical Methods

College of Veterinary Medicine
4700 Hillsborough Street
North Carolina State University
Raleigh, NC 27606
Tel: 919-513-6202
Fax: 919-513-6464
Email: Michael_Dykstra@ncsu.edu

Agar Embedment of Cell Suspensions or Subcellular Particulates for Transmission Electron Microscopy

(from: Dykstra, M.J. 1993. *A Manual of Applied Techniques for Biological Electron Microscopy*, Plenum Press, NY)

1. Applications and Objectives: This technique may be applied to non-adherent mammalian cell cultures, cell suspensions of various types (free-living protozoans, bacteria, disaggregated tissues), or subcellular fractions or organelles large enough to be pelleted by clinical centrifuges or microfuges. This procedure provides a surrounding matrix to suspended cells or subcellular materials so that they can be handled similarly to tissue pieces during subsequent processing steps.

2. Materials Needed:

specimens in primary aldehyde fixative solution
3-4% water agar
Pasteur pipets
centrifuge tubes (preferably polypropylene microfuge tubes)
razor blades
applicator sticks

3. Procedure:

- 3.1. Add 0.3-0.4 g agar to 10 ml of distilled water in a 15-20 ml shell vial and heat on a hot plate in a 50 ml beaker containing distilled water until the agar dissolves.
- 3.2. After the cells or particulates have been in the primary fixative at least 1 hr at room temperature, pellet the sample with a centrifuge adjusted for an appropriate relative centrifugal force (rcf) selected from Table 1.

Table 1:**Sample Centrifugation**

<u>Cell Type</u>	<u>Fixed</u>	<u>Unfixed</u>	<u>Recommended rcf</u>
Protozoans (4-50 μm)	**		250-300
Protozoans		**	300-2000
Mammalian cells (10-20 μm)	**		250-300
Mammalian cells	**		2000-2500
Bacteria (0.5-2 μm)	**		6000-8000
Bacteria	**		9000-9500
Mitochondria, chloroplasts	**	**	6000-9000

- 3.3. Carefully remove the fixative from the pelleted sample with a Pasteur pipet, taking care not to disturb the pellet. Replace with approximately 1 ml of an appropriate buffer. Resuspend the pellet by vortexing to avoid the potential shearing action of vigorous pipetting of particulates with Pasteur pipets. Transfer the resuspended sample to a 1.5 ml microfuge tube, taking care to pipet gently. After 15 min, pellet the sample and resuspend it in fresh buffer. Incubate an additional 15 min.
- 3.4. Pellet the sample and remove the buffer. Heat a clean Pasteur pipet by drawing up heated water several times from the beaker containing the vial of molten water agar. Then, remove approximately 1 ml of molten agar from the vial of agar with the pipet, place the pipet tip at the bottom of the pelleted sample in the microfuge tube and expel the agar. Next, quickly place the microfuge tube into a microfuge already containing a balance tube with 1 ml of water and pellet the suspension at an appropriate rcf for 30 sec.
- 3.5. Remove the microfuge tube containing the agarized sample and wait until the agar has solidified and become translucent. To speed up this step, the sample can be placed at 4° C.
- 3.6. With a single-edged razor blade, carefully slice the end off the microfuge tube just above the agarized pellet. Cut into 1-mm-thin slices. Use an applicator stick to transfer the agarized sample slices to a vial containing osmium and buffer.
- 3.7. Further processing steps can be carried out by gently pipetting fluids in and out of the processing vial as would be appropriate with slices of tissue.

4. Results Expected: This method provides slices containing agar-embedded particulates that can subsequently be handled like slices of tissues, thus avoiding centrifugation throughout the entire processing schedule.

5. Cautionary Statements: If a microfuge is not available, this technique can be performed in larger centrifuge tubes utilizing a clinical centrifuge, and the agarized pellet can be removed by using an applicator stick to remove the whole volume of agar from which the tip containing the sample can then be cut.

A sample that must be spun down with an ultracentrifuge cannot be handled in this fashion since the molten agar would solidify long before sufficient speed was obtained. In that case, gently remove the buffer from the ultracentrifuged sample and add droplets of molten agar (2-3 X the sample volume) to the sample and quickly stir with a pipet tip or applicator stick to suspend the sample in a minimum volume of the agar before it solidifies. After it has cooled, remove the droplet of agarized sample and slice it into 1-mm-thick pieces for subsequent processing. The sample will not be as concentrated as those prepared with the microfuge, but will typically be useable.

When pipetting samples (which should be done as little as possible, particularly with larger cells), remember that cells can suffer shearing artifacts if pipetting is too vigorous. In addition, cells post-fixed with osmium are much more prone to damage since they are more brittle than aldehyde-fixed cells.

It is necessary to pellet the samples in molten agar within 30-60 sec to prevent shearing artifacts caused by the agar solidifying during the centrifuge run.