Preparing Tissue Culture Cells for Paraffin-Embedding

University of Virginia Biorepository & Tissue Research Facility (BTRF)

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This protocol describes the initial steps in preparing cell culture for formalin-fixation and paraffin-embedding. The end result is a cell culture preparation that mimics the histologic processing of tissue samples, allowing the cell culture to be used as controls for tissue immunohistochemistry and *in situ* hybridization.

The volume of the packed cell pellet ideally needs to be approximately 0.5 mL. This requires approximately four 75 cm^2 sized flasks, or two 150 cm^2 flasks of near-confluent cell culture. Less material will result in a size-limited preparation, but this may be sufficient of only a few procedures are expected to be run.

For adherent monolayer cells, do not trypsinize, as this may destroy cell-surface protein markers. Working quickly, pull the flasks from the incubator, and scrape the cells into the media. Transfer to a sterile 50 mL polypropylene centrifuge tube. Spin at room temperature for five minutes in swinging bucket centrifuge (setting 3 for 5 minutes in a standard clinical centrifuge, or approximately 200 x g).

Aspirate media off cell pellet. Very slowly, add 20 ml neutral buffered formalin or zinc formalin (4º C) (contact the BTRF if you require this reagent), letting it flow gently down the side of the tube, in order not to disturb the pellet. You may re-centrifuge if the cell pellet is disturbed.

The cells need to fix overnight at 4º C in formalin. You may bring the cell preparation the day of preparation, or after overnight incubation. If delivery of the preparation cannot be made within 24 hours of the start of fixation, remove the formalin and replace with 20 ml of 70% EtOH WITHOUT resuspending the pellet. This will act as a non-crosslinking preservative, and the cells can be kept this way indefinitely at 4 º C. Do NOT freeze the cells.

The BTRF will process the cell pellet into a paraffin block that can ultimately be used for making histologic sections.