

Frozen Blocks of Cells and/or Embryoid Bodies for Immunostaining

(DRG - 09/03/2009)

Materials:

Plastic Cryomolds for making blocks
OCT (Optimal Cutting Temperature) solution and/or TFM (Tissue Freezing Medium)
Trypsin
Cell Culture Medium
PBS, 1x Sterile
15 ml conical tubes
10 ml Serological Pipettes

Protocol:

- 1) Label cryomolds for each sample then squirt a small amount (~200 ul) colored TFM into each cryomold and allow them to sit at room temp. until the OCT is level. Place in the -80 deg. C freezer for 5 to 10 minutes to freeze the bottom layer.

- 2a) Detach cells from the plate using and make a cell pellet following standard cell culture methods using Trypsin, the appropriate cell culture Medium, PBS and 15 ml tubes.

- or

- 2b) Transfer the embryoid bodies (EBs) from the plate to the 15 ml conical tube using the 10 ml serological pipette and allow 3-5 minutes for the EBs to settle to the bottom.

- 3) Suction off the supernatant and leaving ~50 to 100 ul in the tube with the cells/EBs.

- 4) Cut a bit of the end off of a few 1,000 ul tip to blunt the ends. Bring one OCT block from the -80 deg. C freezer at a time to keep the bottom layers from thawing. Quickly, add ~300-500 ul clear OCT to each block one at a time, followed by the cells/EBs. Use a 200 ul pipette tip to make a small swirl of cells in the middle of the OCT.

- 5) Place the new cell/EB blocks in the -80 deg. C freezer and move on to the next block.

- 6) Once all of the blocks have hardened following the addition of the cells/EBs, open the - 80 deg. C freezer and add enough colored TFM over the top of the white OCT to cover the white layer and allow the blocks to harden.

- 7) Wrap each block in a labeled aluminum foil square, approximately 5" x 5". Then store in a box in the -80 freezer.