Preparing Gel Block from Cell Lines or Cell Suspensions

Material need:

- 4% Neutral Buffered Formalin or Paraformaldehyde (recommended if use fluorescence detection methods) depend on research purpose.
- Centrifuge and centrifuge tubes
- Histo-Gel(Thermo Scientific, cat#: HG-4000-012) or 2% agarose in PBS

To make up a solution of 2% agarose in PBS:

a. Mix 2g of agarose with 100ml 1xPBS (pH7.4). Dissolve in hood.
b. Microwave the mixture for 30 seconds intervals until there are no visible agarose crystals.
   Note: keep the cap untightened during microwave.
c. Seal the container to prevent the gel from drying due to evaporation.
d. Store the 2% agarose gel in 4C fridge. The gel could be reused within a few months if it is properly stored.

1. Harvesting and fixation of the cells

For suspension cells:

a. Transfer the culture cells in a 15 ml/50 ml centrifuge tube depends on the total volume of the culture and Centrifuge to pellet the cell for 5 minutes at ~200g
b. Aspirate the supernatant and resuspend the cell with ice-cold PBS
c. Centrifuge to pellet the cell for 5 minutes at ~200g again
d. Discard the supernatant
   Note: Resuspend cells with residual PBS and proceed with step 2(Embedding) if need frozen procedure. Your cells are still alive and biochemically active at this point.
e. Resuspend cell with adequate volume of fixative to the cell pellet.
   Note: the volume of fixative added to the cells depends on the final residual volume after the supernatant is discarded. As a general principle, the fixative:sample ratio should be >15:1~20:1. i.e., for a residual volume of 200ul pellete+ PBS, 5 ml fixative should be more than that ratio.
f. Fix the cell at room temperature for 15 minutes.
g. Centrifuge to pellet the cell for 5 minutes at ~200g again.
h. Discard the supernatant and resuspend cell with residual fixative.

For adhesive cells:

a. Aspirate the culture media and rinse the cells with ice-cold PBS
   Note: Skip step b and proceed with step c(harvest) if need frozen procedure. So, your cells are still alive and biochemically active at this point.
b. Aspirate the PBS, add adequate volume of fixative (10ml fixative for a 10cm culture plate or 20 ml foe 15cm culture plate) and fix the cells in room temperature for 15 minutes.
c. Harvest the adhesive cells by scraping and transfer the cells in fixative to 15ml/50 ml centrifuge tube. Rinse the residual cells left behind with a few ml of fixative or cold PBS for frozen procedure. Transfer the residual cells into the same centrifuge tub. Do not trypsinize to harvest adhesive cells, as this may destroy cell-surface protein markers
d. Centrifuge to pellet the cells for 5 minutes at ~200g.
e. Discard the supernatant and resuspend cells with residual fixative or PBS for frozen procedure.

2. Suspension Embedding the cell Pellet with Histogel or 2% agarose gel:

a. Transfer the above cell suspension to 1.5 ml Eppendorf tube (estimate the total volume of the concentrated cell suspension).
b. Melt the Histogel or 2% agarose gel by briefly microwave after harvesting the cells or in a 65˚C water bath before harvesting the cells. Mix well before use.
c. Add twice volume of Histogel or 2% agarose gel to the concentrated cell suspension, cap the tube and briefly vertex to mix the gel with cell well.
d. Optional: Invert "capped" tube to let the molten gel-cells mixture flow onto the top part of the tube to get a gel cylinder. Otherwise, you will yield a gel cone.
e. Place the caped tube/inverted capped tube with the still molten gel-cell mixture at room temperature until it is solidified (put on ice to accelerate the process, recommended for frozen procedure).

3. Preparation of Gel-Cell blocks for histopathology assay:

a. Cut off the bottom of Eppendorf tube with a sharp blade. Open the cap carefully and gently extract the gel cylinder/cone by pushing it out from the open bottom with a stick or pipette tip.
b. For frozen procedure, please embed the gel in appropriate frozen embedding media such as O.C.T. compound. For frozen embedding procedures, see **Sample preparation instruction for frozen embedding.**
c. At this point you can cut the agar piece in two with a razor blade if the smallest dimension of the gel cylinder is more than 4mm to make its smallest dimension less than 4mm.
d. Put the gel block in labeled cassette and fix the gel with the same fixative used above for 16-24 hours if you prefer paraffin process. Transfer the cassettes into 70% Ethanol and it is ready to submit to pathology lab for processing.