PROTOCOL

SAMPLE PREPARATION FOR HISTOLOGICAL ANALYSIS

RECOMMENDED MATERIALS AND REAGENTS

- Cells cultured with VitroGel system
- DPBS (no calcium, no magnesium)
- Cassettes
- 2% Paraformaldehyde
- Paraffin, xylene, fixation solution (10% buffered formalin)
- Ethanol (50-100%)
- Water bath

PROTOCOLS: (using 24 well-plate, 300 µL gel/well as an example)

Protocol 1:

1. Remove the cover media from the top of the hydrogel and wash with 1 mL DPBS
2. Add 1 ml of 2% paraformaldehyde (PFA) to fix the cells in hydrogel for 30 minutes at room temperature. Do not place it on a rocker or any moving platform as it will disturb the hydrogel layer.
   
   **Note:** Occasionally, PFA may cause depolymerization of the hydrogel, the addition of 0.1% glutaraldehyde (GA) solves this issue. On the other hand, glutaraldehyde may produce higher autofluorescence, so it is important that the quenching step is optimized for each particular protocol.

3. Remove PFA and wash carefully with 1 ml of DPBS: add 1 mL DPBS to the top of hydrogel and wait 1-3 minutes before discarding. Wash 3 times.
4. Transfer the fixed hydrogel in a cassette and keep in 70% Ethanol (EtOH) for further paraffin embedding.
5. Paraffin embedding:
   - 70% Ethanol, two changes, 1 hour each
   - 80% Ethanol, one change, 1 hour
   - 95% Ethanol, one change, 1 hour
   - 100% Ethanol, three changes, 1.5 hours each
   - Xylene or xylene substitute (i.e. Clear Rite 3), three changes, 1.5 hours each
   - Paraffin wax (58-60 °C), two changes, 2 hours each
   - Embedding tissues into paraffin blocks

6. Trim paraffin blocks as necessary and cut at 3-10 µm (5 µm is commonly used).
7. Place paraffin ribbon in water bath at about 40-45 °C.
8. Mount sections onto slides.
9. Allow sections to air dry for 30 minutes and then bake in 45-50 °C oven overnight.

**Note:** NEVER allow baking temperature to go higher than 50 °C for sections thicker than 25µm. Otherwise sections may crack, especially 25-50 µm thick sections, and result in sections falling off slides during staining.

Additional Materials:
- O.C.T. Compound (Tissue-Tek 4583)
- Cryomold (Tissue-tek cryomold standard 4557, 25x20x5mm)

1. Remove the cover media from the top of the hydrogel and wash with 1 mL DPBS
2. Add 1 mL of 2% paraformaldehyde (PFA) to fix the cells in hydrogel for 30 minutes at room temperature. Do not place it on a rocker or any moving platform as it will disturb the hydrogel layer.
3. Remove PFA and wash carefully with 1 mL of DPBS: add 1 mL DPBS to the top of hydrogel and wait 1-3 minutes before discarding. Wash 3 times.
4. Transfer the fixed hydrogel in a cassette and keep in 70% Ethanol (EtOH) for at least 2 hours.
5. Add a small amount of O.C.T. compound into a cryomold and place the Cryomold on ice for 5 -10 minutes.
6. Carefully transfer the hydrogel into the Cryomold with O.C.T. compound base.
7. Add more O.C.T. compound on top of the hydrogel in the Cryomold to form a sandwich
8. Keep the cryomold at -20 °C for 15-30 minutes. The sample is ready for frozen-section.

Protocol 3: Using Histogel for sample preparation

Additional Materials:
- Histogel (Thermo Scientific Cat# HG4000012)
- 0.5% Eosin in 70% Ethanol
- Cryomold (Tissue-tek cryomold standard 4557, 25x20x5mm)
- 10 % Neutral Buffered Formalin

1. Warm the Histogel in 65 °C water bath for 1 to 2 hours until it liquefies.
2. Remove the cover media from the top of the hydrogel and wash with 1 mL DPBS
3. Add 1 mL of 2% paraformaldehyde (PFA) to fix the cells in hydrogel for 30 minutes at room temperature. Do not place it on a rocker or any moving platform as it will disturb the hydrogel layer.
4. Remove PFA and wash carefully with 1 mL of DPBS: add 1 mL DPBS to the top of hydrogel and wait 1-3 minutes before discarding. Wash 3 times.
5. Place the Cryomolds on ice.
6. Add 150 µL warm Histogel into a cryomold and gently spread it with a pipet tip. Place the Cryomold back on ice for 5 -10 minutes.
7. Transfer the hydrogel into the Cryomold with Histogel base.
8. Add 150 µL warm Histogel on top of the hydrogel in the Cryomold to form a sandwich
9. Cool on ice for 10-15 minutes.
10. Transfer the Histogel block into a tissue cassette
11. Place the tissue cassette in 10% Formalin for 16-20 hours.
12. Transfer the cassette to a container with 0.5% Eosin in 70% ethanol for 1-2 days
13. After 2 days, store the cassettes in 70% ethanol until they can be processed for paraffin embedding.