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 auto-fluorescence, 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.

Protocol 2: Using O.C.T. compound for Cryo-sample preparation

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.

Corporate Headquarters
TheWell Bioscience Inc.
675 US Highway 1
North Brunswick, NJ 08902 US TF: +1 866.3D.CELLS TEL: +1 973.855.4955 | support@thewellbio.com | www.thewellbio.com