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Hydrogel Embedding and Sectioning for Histology Analysis Protocol

Description

This protocol aims to provide instructions on preparing cell-seeded hydrogel sections for cryo- or paraffin-based histology analysis assays.

Materials Needed

- Cell-seeded hydrogels (i.e. PureCol®)
- 4% paraformaldehyde (ThermoFisher, Cat # 047392-9M)
- 1X PBS
- 30 % Sucrose solution
- Tissue-Tek™ CRYO-OCT compound (Fisher Scientific, Cat #14-373-65)
- Disposable base molds (Fisher Scientific, Cat #22-363-553)
- Tissue processing cassette (Fisher Scientific, Cat #15-182-701A)
- Embedding cassette metal base mold (Fisher Scientific, Cat #64-010-X)
- Biopsy foam pad (Fisher Scientific, Cat #22-038-221)
- Low profile microtome blades (Fisher Scientific, Cat #3050822)
- Microscope adhesion slides (Fisher Scientific, Cat #12-545-78)
- Microscope cover glass (Fisher Scientific, Cat #50-136-7540)
- Permanent marker
- Xylene (Sigma-Aldrich, Cat # 534056)
- Cold acetone
- Tissue processor
- Tissue embedder
- Microtome/Cryo-microtome
- Water bath
- Dry ice
- -80 °C freezer
- Cell culture plates
- Centrifuge tubes
- Serological pipettes

Procedure

Method 1: Cryo-embedding and sectioning

Fixation

1. Remove cell culture medium and add 4% formaldehyde solution to the hydrogel wells and incubate at room temperature for 1 hour to fix the cells.

2. Remove the formaldehyde solution and add the 30% sucrose solution to the hydrogels and incubate the samples overnight.

3. Discard the sucrose and wash the hydrogels with 1X PBS three times.

Cryo-embedding

4. Use the permanent marker to label the disposable tissue mold with sample information.

5. Transfer the fixed gels into the plastic disposable tissue mold using a spatula.

6. Aspirate/wipe off the remaining PBS and briefly air dry the fixed sample for 5 min.

7. Dispense enough OCT compound to cover the fixed hydrogel sample in the plastic mold.

8. Place the OCT-covered samples on dry ice until the OCT compound freezes and solidifies.

9. Transfer the samples into a -80 °C freezer and freeze overnight. *The samples can be stored in the freezer for long-term preservation (> 6 months).

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Cryo-sectioning

10. Adjust the temperature of the cryotome to -20 °C and ensure the chamber is fully cooled down before use.

11. Label the glass slides with the permanent marker.

12. Remove the sample from the mold and glue it to the sample holder for the cryotome using OCT compound. Wait until the OCT compound freezes and ensure the sample is tightly attached to the holder.

13. Load the sample holder to the sample stage and tighten the screws.

14. Install the tissue blade to the blade slot in the cryotome. ***Be extra careful with the blade, it is super sharp and should be handled carefully.***

15. Set the desired section thickness on the cryotome and section the sample block. *For hydrogels we recommend 5 to 10 µm thickness.

16. Once a complete section is obtained, affix it to a dry glass slide. The higher temperature of the glass slide will help the cyro-sections attach to the slide easily.

17. Soak the slides in cold acetone for 5 min to wash off the OCT compound and increase the adhesion of the hydrogel sections. Dry the slides on a rack after soaking.

18. Transfer the sections into a slide box and store the box in the -80 °C freezer for long-term storage. The slides are ready for histology staining.

19. Remove the blades from the cryotome and clean the instrument.

Method 2: Paraffin-embedding and sectioning

Fixation

1. Remove cell culture medium and add 4% formaldehyde solution to the hydrogel wells and incubate at room temperature for 1 hour to fix the cells.

2. Remove the formaldehyde solution and wash the hydrogels with 1X PBS three times. Samples can be stored in PBS at 4 °C before embedding.

Processing

1. Turn on the tissue processor to warm it up about 2 hours before loading samples. ***Refer to manufacturer's instructions.***

   *Note: Make sure to check the levels of all processing reagents and waste containers. Refill the reagents or empty the waste if needed.

2. Label mega-cassettes for samples as needed using the permanent marker.

3. Place two biopsy foam pads inside the mega-cassette and place the sample between the foam pads.

4. Place the cassettes in a 500 mL beaker and pour PBS into the beaker to cover the cassettes.

5. Load samples into the instrument and start processing. Processing can take up to 12 hours depending on the instrument model.

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Embedding

6. Turn on tissue embedder and allow it to run for about 1 hour before use to melt the wax.
7. Remove processed samples from tissue processor and place them on paper towels to remove excess melted wax.
8. Transfer the cassettes into the warm compartment of the tissue embedder.
9. Clean the tissue processor and initiate the automatic clean cycle.
10. On the tissue embedder, open the cassette and remove the lid and top biopsy foam pad.
11. Fill about 1/3 of the base mold with melted wax/paraffin and carefully transfer the gel into the mold. Push the gel to the bottom of the mold using a spatula.
12. Move the mold to the freezing pad and allow the wax to solidify and secure the gel in place at the bottom.
13. Fill the rest of the volume of the mold with melted wax.
14. Place the cassette case on top of the mold and move the mold to the freezing pad to solidify the wax completely (> 30 minutes).
15. Gently remove blocks from molds after all wax is solidified. The blocks are now ready for sectioning.

Sectioning

16. Label the glass slides with the permanent marker.
17. Fill a water bath with DI water and warm up to 60 °C.
18. Turn on the microtome and set section thickness. *We recommend 5 to 10 µm for hydrogels.
19. Install the section blade onto the blade slot and secure levers on the cutting block.
20. Install the paraffin-embedded sample block on to the sample holder and adjust the position to ensure smooth sectioning.
21. Rotate the handle on the microtome to section the block.
22. Collect and transfer the good sections onto the water bath. The sections will float on the water.
23. Place the slide under sections and gently lift it up to attach the sections to the slide.
24. Use Kimwipes to wipe off the excessive water remaining around the sections.
25. After completing the sectioning, bake the slides in a dry oven at 60 °C for 30 minutes to enhance their adhesion to the slides.
26. The slides can be stored at room temperature before proceeding to histology analysis.
27. Remove the blades from the microtome and clean up the instrument.

Notes:

1. IMPORTANT: Make sure all levers are locked before removing blades from microtome.

2. Warm the paraffin block as needed by tapping it briefly on the warm water to facilitate sectioning.

