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Hydrogel H&E Staining Protocol

Description

Hematoxylin and eosin (H&E) staining is a widely used technique in histology and pathology for the visualization of tissue architecture. Hematoxylin stains cell nuclei blue or purple, while eosin stains the cytoplasm and extracellular matrix pink or red. This contrast allows for detailed examination of tissue morphology, making H&E staining an essential tool for diagnosing diseases, studying tissue structure, and conducting research in various biomedical fields.

This protocol provides instructions on performing the H&E staining on mouse cells cultured in 3D hydrogels for histology analysis, that have been paraffin or frozen-prepared.

Materials Needed

- Prepared paraffin or frozen-sections
- Mouse and Rabbit Specific HRP/DAB Detection IHC kit (Abcam, Cat#ab64264)
- Xylene (Sigma-Aldrich, Cat # 534056)
- Ethanol
- 10 mM Sodium Citrate buffer
- 1 × PBS
- Hydrochloride acid (HCl)
- Permount mounting medium (Fisher Scientific, Cat # SP15-100)
- Cover glass
- Mounting medium
- PAP pen
- Dry oven
- Centrifuge tubes
- Serological pipettes
- Staining jar
- Staining tray/chamber
- Micropipette and tips
- Nail polish
- Microscope

Procedure

For paraffin sections:
For frozen sections, skip to step 10.

1. Incubate sections in xylene #1 for 5 min.
2. Transfer sections into xylene #2 and soak for 5 minutes to completely remove the paraffin.
3. Soak sections in 100% Ethanol #1 for 5 minutes.
4. Transfer sections into 100% Ethanol #2 for 5 minutes to remove xylene.
5. Perform serial rehydration by soaking the sections in 90%, 70%, 50%, and 30% Ethanol for 5 minutes in each wash.
6. Soak sections PBS #1 for 5 min.
7. Soak sections PBS #2 for 5 min.
8. Rinse sections in a PBS.
9. Perform **antigen retrieval**:

   9.1 Add 10 mM Sodium Citrate buffer in the staining jar and put the microscope slides in the jar, making sure the slides are fully covered by the buffer.
   9.2 Place the jar in a water bath and heat up the water to 98°C-100°C. Once the water starts boiling, incubate the samples for 10 minutes.
   9.3 Cool the samples in the buffer to room temperature.

   Proceed to Step 10 after antigen retrieval.

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For frozen sections:

10. Bake slides in an oven set to 37 °C for 30 minutes.
11. Circle the areas for staining on the slides using a PAP pen.
12. Soak slides in PBS for 30 min for rehydration in a jar.
13. Take slides out and gently shake off PBS on Kimwipes.
14. Add enough drops of Hydrogen Peroxide Block to cover the sections. Incubate for 10 minutes at room temperature.
15. Wash 2 times in PBS and soak for 5 minutes for each wash, use fresh PBS for each wash.
16. Apply the Protein Block and incubate the slides for 10 minutes at room temperature to block nonspecific background staining. Wash the slides once with PBS.
17. Apply the primary antibody (1:200 diluted in PBS) and incubate for 2 hours at room temperature or overnight at 4 °C.
18. Wash the slides 4 times in PBS with 5 minutes soaking in fresh PBS for each wash.
19. Gently shake off the antibody solution on Kimwipes.
20. Apply Biotinylated Goat Anti-Polyvalent and incubate for 10 minutes at room temperature.
21. Wash the slides 4 times in PBS, for 5 minutes intervals, soaking in fresh PBS for each wash.
22. Gently shake off antibody solution on Kimwipes.
23. Apply Streptavidin Peroxidase and incubate for 10 minutes at room temperature.
24. Dip slides 4 times in PBS.
25. Add 30 µL (1 drop) DAB Chromogen to 1.5 mL (50 drops) of DAB Substrate, mix by swirling and apply to tissue. Incubate for 10 min.
26. Replace the PBS in Step 24 with a new one and dip slides 4 times in the new PBS.
27. Add Hematoxylin to the staining area and incubate for 5 min.
    *** Note:** Filter fresh Hematoxylin (CAT Hematoxylin) using 0.2 µm syringe filter before use.
28. Gently shake off Hematoxylin on Kimwipes and wash the back of the slides under running tap water.
29. Differentiate sections with 1% HCl in 70% ethanol. Quickly dip slides in the solution, gently shake off the solution on Kimwipes and check under microscope. If needed, put slides back into Hematoxylin for further staining (~3 minutes).
30. After complete staining and differentiation, soak slides in DI water for 15 minutes.
31. Take slides out and perform serial dehydration:
    - Soak in 30% ethanol for 30 s.
    - Soak in 50% ethanol for 30 s.
    - Soak in 70% ethanol for 30 s.
32. Quickly dip slides in 95% ethanol twice.

33. Gently shake off ethanol on Kimwipes.

34. Mount the slides with coverslips using the Permount mounting medium.

35. Dry the slides for 2 hours or overnight. *Seal the coverslip edges with nail polish to preserve the slides for long-term storage.

36. Image the slides with an inverted microscope.

