In Vitro Application

Procedure for Preparation and Paraffin Embedding of LifeGel Samples from 96-Well Plates for Immunohistochemistry (IHC)

PROTOCOL:

Sample preparation and fixation

1. **Remove Culture Medium**
   Carefully aspirate all culture medium from each well of the 96-well plate.
2. **Wash with PBS**
   Rinse each well twice with phosphate-buffered saline (PBS) to remove residual media.
3. **Fixation**
   Add 200 µL of 10% neutral buffered formalin (or 4% paraformaldehyde [PFA]) to each well to fix the samples.
4. **Incubation**
   Incubate the plate at room temperature for 30 minutes to ensure proper fixation.
5. **Remove Fixative**
   Aspirate the fixative solution completely from each well.
6. **PBS Wash**
   Wash the wells 2–3 times with PBS to remove any remaining fixative.
7. **Contrast Staining (Optional)**
   Add 50 µL of 0.2% nigrosin solution in PBS to each well for contrast enhancement.
8. **Stain Incubation**
   Incubate for 5 minutes (extend up to 15 minutes if stronger contrast is needed).
9. **Post-Staining Wash**
   Wash wells 2–3 times with PBS to remove excess stain.
10. **Drying Preparation**
    Aspirate as much PBS as possible from each well to minimize residual moisture.
11. **Drying Step**
    Place the plate on a heat block or heating plate at low heat (~30 minutes) to evaporate remaining liquid before embedding.

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In Vitro Application

PROTOCOL:

Sample preparation and fixation

12. Add Agar to Wells

While the plate remains on the heating surface, dispense approximately 50 µL of 1% agar solution in PBS into each well. This prevents the agar from solidifying prematurely.

13. Solidify Agar

Allow the agar to solidify on the heating plate for 10–15 minutes.

14. Cool to Room Temperature

Remove the plate from the heat and allow it to cool gradually to room temperature.

15. Extract Embedded Plugs

Using a rounded-tip spatula, carefully extract the agar-embedded plugs (containing LifeGel) from each well.

16. Stabilize Samples

Coat each extracted plug with a thin layer of molten agar to reinforce the structure and maintain sample integrity.

17. Begin Dehydration

Immediately transfer the stabilized plugs into 70% ethanol for initial dehydration and short-term storage prior to further processing (e.g., paraffin embedding).

In Vitro Application

PROTOCOL:

Dehydration and Paraffin Embedding Protocol

1. **Initial Dehydration**:
   Submerge agarose plugs in 80% ethanol for 1.5 hours.
2. **Overnight Ethanol Incubation**:
   Transfer the samples to 96% ethanol and incubate overnight (12 hours).
3. **Absolute Ethanol Dehydration**:
   Sequentially incubate samples in three changes of 100% ethanol, each for 30 minutes:
   - 100% EtOH (I) – 30 min
   - 100% EtOH (II) – 30 min
   - 100% EtOH (III) – 30 min
4. **Clearing with Xylene**:
   Transfer the samples into three changes of xylene, 30 minutes each:
   - Xylene (I) – 30 min
   - Xylene (II) – 30 min
   - Xylene (III) – 30 min
5. **Intermediate Infiltration**:
   Incubate the samples in a xylene/paraffin mixture for 1.5 hours.
6. **Paraffin Infiltration**:
   Transfer to fresh paraffin (II) and incubate for 1.5 hours.
   Follow with a final incubation in fresh paraffin (III) overnight.
7. **Embedding:**
   Embed the agarose plugs in paraffin using appropriate embedding molds or tissue cassettes. Once solidified, section the paraffin blocks using a microtome (recommended thickness: 4–6 µm).

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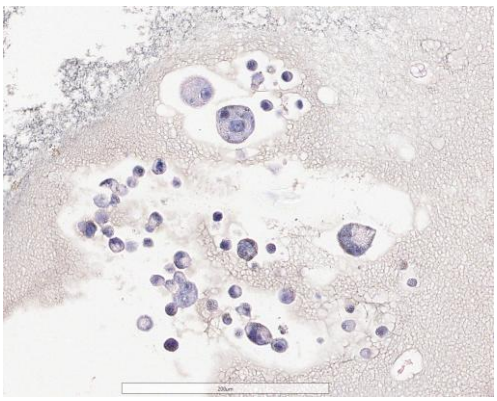

Fig. 1 Immunohistochemical staining for N-cadherin of SKBR3 cells grown in spheroind on LifeGell plates, parafin embanded and sectioned using microtome.

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