Spheroid Processing and Embedding for Histology

Guidelines for Use

CORNING

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Materials and Reagents

- 96- or 384-well spheroid microplates (Corning Cat. No. 4520 or 3830) containing multicellular spheroids ready for histological analysis
- Phosphate buffered saline (PBS) pH 7.4 (Corning Cat. No. 21-040-CM)
- 4% paraformaldehyde (PFA) in PBS pH 7.4
- HistoGel™ (Richard-Allan Scientific™ Cat. No. HG-4000-012)
- Ethanol or reagent grade alcohol (70%, 95%, and 100%)
- Xylene (or xylene substitute suitable for tissue clearing)
- Paraffin or Paraplast® (heated to appropriate melting temperature)
- Axygen® wide bore pipet tips (Corning Cat. No. TF-205-WB-R-S)
- Embedding cassettes
- Lens paper
- Disposable biopsy mold (optional)
- Fine forceps

Procedure

Fixation

1. Remove spheroid microplate from incubator. Pipet or aspirate as much media as possible without disturbing the spheroids. Rinse cells with PBS.
2. Remove PBS and fix the cells by adding enough cold 4% PFA to cover the spheroids. Fix for 30 minutes to 1 hour at room temperature.
3. Rinse off the 4% PFA with three changes of PBS.

Transfer to HistoGel Embedding Medium

NOTE: It is possible to embed cultures without agarose-based medium, but is considerably more challenging and time-consuming to handle individual spheroids. Place several spheroids in a square of lens paper, carefully fold to prevent tissue loss, and place into cassette. If processing without agarose gel, less time will be required in each reagent. Use forceps to transfer spheroids from molten paraffin to molds.

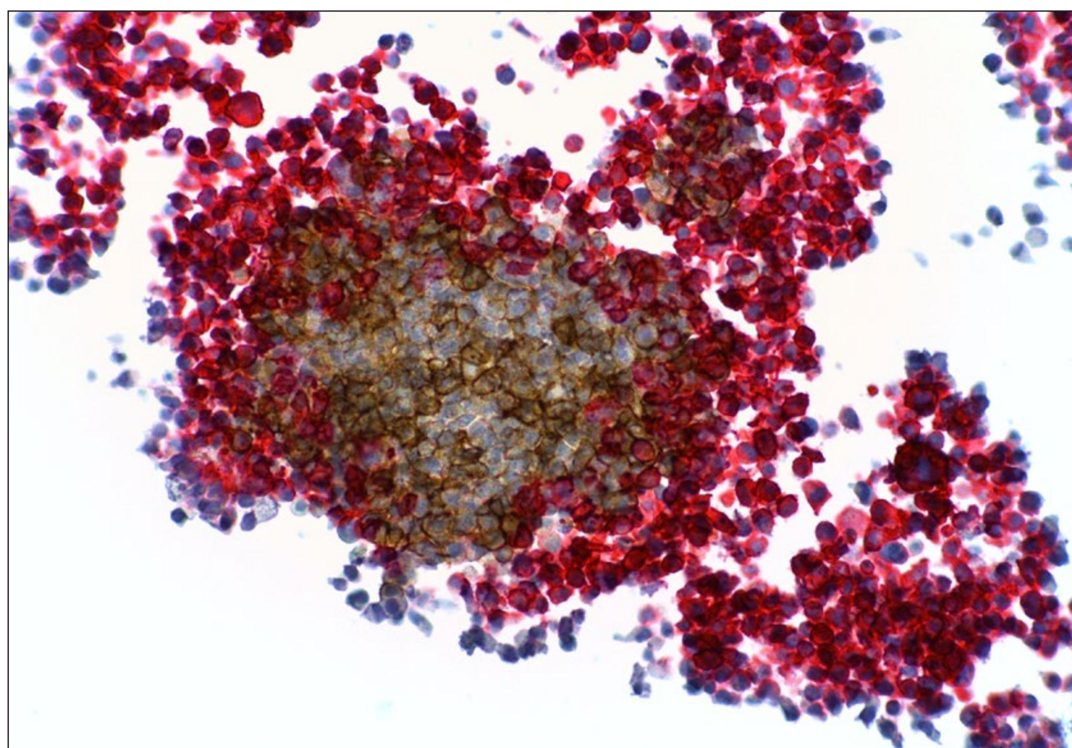
4. Label a tissue cassette with the identification number of the sample using a pencil or chemical-resistant marker.
5. Melt HistoGel according to manufacturer's instructions using microwave, water bath, etc. Keep molten gel at 50°C to 55°C until use.
6. Using wide bore pipet tips to avoid damaging samples, transfer several spheroids into the center of a disposable biopsy mold or onto a small area of lens paper (<1 cm^2). Be careful not to transfer excess liquid.
7. Cover spheroids with a few drops of molten HistoGel, then transfer to cold surface for rapid solidification (2 to 5 minutes). Ensure that spheroids are centered within block (i.e., not on the outer edges).
8. Remove solidified HistoGel from paper or mold using forceps, transfer to labeled processing cassettes, then place in container of 70% alcohol for processing.

Processing, Embedding, Sectioning, and Staining

9. Process into paraffin using a protocol suitable for small samples (e.g., biopsy protocol). Success was achieved using an automated tissue processor and pressure/vacuum at 15 minutes per station.

   NOTE: Longer processing times may improve results and prevent shrinking/hardening of agarose, as may a more gradual increase to 70% alcohol in Step 8 (e.g., 10%, 30%, 50%)[1]. Final volume of HistoGel cube will also impact processing time. Processing replicate samples in separate HistoGel cubes is advised in case of hardening or shrinking, which can adversely impact sectioning.

10. Upon completion of paraffin infiltration, transfer cassettes to molten paraffin in embedding center. Embed samples in desired orientation in embedding molds, then transfer to cold plate.
11. Once embedded, samples may be sectioned, stained, and immunostained with routine protocols. Automated deparaffinization, H&E, heat induced epitope retrieval, and room temperature immunostaining have all been performed with success using standard protocols.



200X microphotograph of 5 micron section of spheroid culture processed using this protocol. A549 cells co-cultured with natural killer cells and dual immunostained for E-cadherin (brown) and CD45 (red).

Reference

1. Histonet Mail Archive, “re: [Histonet] Histogel”, <http://lists.utsouthwestern.edu/pipermail/histonet/2014-January/069494.htmll>, 1/20/2014.

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