Special Techniques Cell Pellet Protocol

This protocol is modified from Joseph Garaots, M.D. and C. E. Lincoln, HT

Reagents needed:

PBS-Calcium Magnesium Free
moats website

Trypsin/EDTA
Gibco Invitrogen Corp
Grand Island NY 14072
www.invitrogen.com
1-888-584-8929
Cat# 25300-054

DMEM/F12
Gibco Invitrogen Corp
Grand Island NY 14072
www.invitrogen.com
1-888-584-8929

Tissue-Tek™ OCT (optional)
VWR International
1310 Goshen Parkway
West Chester, PA 19380
http://www.vwr.com
1-800-932-5000
Cat# 25608-930

US Biotex Biopsy Filter Papers™
www.usbiotex.com
1-800-786-4614

HistoGel™ Kit Specimen processing gel
Kalamazoo, MI
www.rallansci.com
1-800-522-7270
Cat# HG-40000-012

Dilute Eosin
1% aqueous Eosin Y diluted with enough 95% ethanol to make a light pink solution.

Fixatives that may be used:
10% Neutral Buffered Formalin
4% Paraformaldehyde
70% Ethanol

Cell Pellet Preparation

1. Grow cells to confluency on p150 plate.
2. Wash cells in PBS-CMF 2X.
3. Add 2 ml 1X Trypsin/EDTA. Digest for 5 minutes at 37°C.
4. Stop digestion by adding 8 ml media (DMEm/F12).
5. Gently wash cells off plate and transfer by pipette to a 15 ml conical tube.
6. Spin cells at 1000- 12000 rpm at 4°C or room temperature for 5 minutes.
7. Decant supernatant.
8. Gently tap tube to loosen cell pellet.
9. Add 5 ml PBS-CMF to re-suspend the pellet.
10. Add 5 ml of PBS-CMF for a total volume of 10 ml. Repeat spin, decant supernatant and repeat wash.

Frozen Preparation

1. Add Tissue-Tek OCT media to suspend pellet. Freeze on dry ice. For sectioning, slightly warm tube with hand to remove pellet.
2. Immediately mount pellet sample on a chuck for frozen sectioning.

Paraffin Preparation

1. Add 5 ml of fixative with dilute Eosin for coloration and vortex mildly. Fix for 15 minutes. Note: if you wish to mimic optimal tissue fixation for Immunohistochemistry protocols, fix overnight (18-24 hours).
2. Spin at 1800 rpm for 10 minutes at room temperature.
3. Decant supernatant.
4. Add 5 ml of 70% ethanol for 30 minutes and vortex mildly.
5. Spin at 1800 rpm for 10 minutes at room temperature.
6. Decant supernatant.
7. Add 5 ml of 100% ethanol for 30 minutes and vortex mildly. Optional: Overnight incubation in 100% ethanol at 4°C makes a very solid pellet.
8. Spin at 1800 rpm for 10 minutes at room temperature.
9. Decant supernatant.

Cell Pellet Embedding

1. Prepare HistoGel™ per manufacturers directions.
2. Using a clean pipette, add 3-5 ml of liquid HistoGel™. Vortex mildly to distribute and place on ice.
3. Using a clean wooden applicator stick, carefully slide the cell pellet out of tube and into a cassette lined with black biopsy filter paper.
4. Place cassette with pellet in 70% ethanol and submit to histology for processing.

Cell Pellet Processing

1. Program tissue processor for a short schedule (i.e. program for small samples such as biopsies).

2. Process pellet samples and embed in paraffin within 24 hours of preparation.

Special thanks to Lois Annab for reagent references.

Pat Stockton
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