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Title: Fixing Suspension Cells to Slides for Confocal Microscopy

PRINCIPLES OF THE PROCEDURE:

Confocal microscopy allows for high resolution analysis of the distribution of fluorescently-labeled antigens throughout defined regions of a cell or tissue. This technology relies on precision scanning from temporally separated laser sources in order to measure the fluorescence emission from samples that have been labeled with more than one fluorescently-labeled antibody. Therefore, the most accurate images are obtained when samples remain in the same spatial planes during the imaging process. For tissue sections or adherent cells that are growing on a coverslip, this is generally not a problem; however for suspension cells, it is best to fix them to the slide prior to confocal acquisition.

SPECIMEN REQUIREMENTS:

Most viable, suspension cell types will adhere to slides when processed according to this protocol.

CRITERIA FOR SPECIMEN REJECTION:

Suspension cells that are already fixed will not adhere well to slides when using this protocol. Previously fixed cells should not be used.

REAGENTS:

1. Alcian Blue Solution, 1% Aqueous (Electron Microscopy Sciences, Cat# 26026-01)

2. diH_20

3. Phosphate Buffered Saline (PBS) or Hanks' Balanced Salt Solution (HBSS)

4. 2% Formaldehyde Solution in PBS

EQUIPMENT & INSTRUMENTATION

2-Well Chamber Slides (Nunc, Cat# 177380 or equivalent)
Pipettors and Tips
Centrifuge
15mL Tubes or 50mL Tubes
Tube Rack
Centrifuge Adapters for 96-Well Plates
Laboratory Tape
Chronometer or other Time-Telling Device

PROCEDURE:

1. Determine the number of slides you will need for the experiment. Label each slide accordingly. Add 1mL Alcian Blue solution to each well of each chamber slide to be used, and incubate for at least 15 minutes at room temperature. Ensure that the Alcian Blue solution is distributed across the entire surface area of the well. Slides can be incubated with Alcian Blue for longer periods of time, if necessary.

2. After the incubation period has elapsed, remove Alcian Blue from each well, and thoroughly rinse each well with diH_20, until the water runs clear (typically 4 volume exchanges). Cover the slides with their lids to prevent excessive desiccation or deposition of dust. Slides should have a discernable blue cast when viewed obliquely.

3. Harvest suspension cells from their culture vessel into a container of suitable size, and centrifuge at 400g for 5 minutes to pellet the cells.

4. Remove cell culture media supernatant by aspiration or decanting. Resuspend the cells and wash them 2 times with a protein-free buffer such as PBS or HBSS. Centrifuge as in Step 3 after each washing step.

5. Count the cells and adjust to 1 x 10^6 cells/mL in a protein-free buffer. Pipette 1mL of the cell suspension into each chamber of the Alcian Blue pre-treated slides.

6. Affix slides to the centrifuge adapters for 96-well plates, using laboratory tape. Centrifuge at 300rpm (or the lowest setting on your centrifuge) for 3 minutes to gently deposit the cells onto the slide surface. It is important to avoid excessive centrifugation speeds, which can deform the cells.

7. After centrifugation, remove slides from the 96-well plate adapters. Tip the slide 45 degrees and GENTLY remove the buffer supernatant by aspirating with a 1mL pipette from the corner of the slide. Make note of the corner that is used, and perform subsequent wash-step aspirations from the same corner. A hazy monolayer of cells should be apparent to the naked eye when the slide is viewed obliquely. Confirm the cell density and distribution

by low-power light microscopy. Desired cell densities will vary by application but in general, there should be enough cells to expedite confocal image acquisition, but not so many that they are overlapping each other.

8. GENTLY add 1mL of 2% formaldehyde in PBS to the slide by pipetting into the corner identified in Step 7. Incubate slides for 15 minutes to crosslink cell surface molecules to the Alcian Blue treated slides.

9. Remove 2% formaldehyde from the slide chambers using the technique described in Step 7. Wash the slides 2 times with PBS using the technique described in Step 7. Cells are now ready for permeablization and labeling with fluorescently-labeled reagents. Add the aforementioned reagents to the slides in permeablization buffer and execute the protocol for fluorescently labeling cell antigens for confocal microscopy.

PROCEDURAL NOTES:

INTERFERENCES: Protect slides from desiccation, especially after cells have been deposited and fixed to the surface.

QUALITY CONTROL GUIDELINES

N/A

EXPECTED VALUES:

N/A

REPORTING RESULTS & CALCULATIONS

N/A

See reporting of results.

REFERENCES:

N/A

VERSION HISTORY:

Version	Effective Date	Section	Description of Revisions/Justifications
			•
2012.01.23			

Name / Title	signature	Date
Author: name / title Joseph D. Tario, Jr., Pre-Doctoral Research Affiliate		01-23-12
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