CMBL – CRJ/JJR 8/1/07

Protocol: Actin Staining

Materials:

General:

Cells: NIH/3T3 fibroblasts seeded @1,000 to 3,000 cells/cm^2 24 to 48 hrs prior to staining

Coverslips
Kimwipes
Aluminum Foil
Pipets/Pipet aid
Pipet tips/pipetters
Aspirator
Timer
Waste beaker
50 mL centrifuge tube ("formaldehyde waste")
Markers
Gloves

Reagents/ Solutions:

Phosphate-buffered saline (PBS), pH 7.4
3.7 % Formaldehyde in PBS (use methanol-free formaldehyde)
0.1 % Triton X-100 solution (in PBS)
PAP pen (Sigma Aldrich Cat # Z377821)
Vectashield mounting media with DAPI – (Vector Laboratories Cat # H-1200)
Clear fingernail polish

Primary Blocking Solution:
PBS + 1% (w/v) BSA + 0.1% (v/v) Nonidet P40

Alexa Fluor 488 Phalloidin Solution: **protect from light – (**Invitrogen Cat # A12379)
5 uL Alexa Fluor 488 Phalloidin in 200 uL *Primary Blocking Solution* (for each coverslip area)

Procedure:

1. Wash cells 2X with PBS (10 mL per wash) – remove all PBS from around slide after final wash
2. Fix cells: apply 2 mL of 3.7% formaldehyde solution on top of slide for 10 min. @ room temp.
   Note: formaldehyde is toxic; avoid contact with skin, eyes, etc.; dispose of as hazardous waste, do not pour down sink
3. Wash cells 2X with PBS (10mL per wash) – place waste in the "formaldehyde waste" tube
4. Permeablize cells: apply 2 mL of 0.1% Triton X-100 solution on top of slide for 5 min. @ room temp.
5. Wash cells 2X with PBS (10 mL per wash)
6. Make a PAP pen region the on the slide – see instructions/pattern below
7. Wash coverslip area 1X with PBS (~200 uL should cover area)
8. Actin staining: apply 200 uL of *Alexa Fluor 488 Phalloidin Solution* for 20 min. @ room temp
   * **Protect from light** – for all subsequent steps place aluminum foil over culture dish to prevent photobleaching of the *Alexa Fluor 488*
9. Remove the *Phalloidin Solution* and wash the coverslip area 3X with *Primary Blocking Solution*
10. Remove the *Blocking Solution* and add Vectashield mounting medium (25uL per coverslip area)
11. Place a coverslip over the stained area and seal the coverslip by pipetting a bead of clear fingernail polish along the edge of the coverslip.
12. Allow the nail polish to dry before viewing on a fluorescent microscope.

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i. Remove the slide from the culture dish and dry the bottom of the slide with a Kimwipe
ii. Place the slide on the pattern
iii. Use a Kimwipe to wipe dry the area in gray
- do not disturb the white coverslip area you will be staining
iv. Apply the PAP pen along the black square around the coverslip area
v. Dry culture dish, and place slide back in the dish