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Protocol For Labeling Cells for Morphology and Nuclear Analysis (Tx-Red and DAPI Double Labeling).

Summary: This procedure is used to stain fixed cells with both TxRed maleimide and DAPI. Results in a very high contrast whole cell and nuclear staining.

NOTE #1: Need DPBS containing 0.01% NaN_3 to prevent cell growth

1. You need Tx-Red Maleimide (5 mg/mL in DMF) and DAPI (1 mg/mL) in EtOH or DMSO and 0.1% Tx-100 in DPBS
2. Fix cells with formaldehyde (1%) in PBS or MBS in MTSB if using GFP cells.
3. Rinse with PBS/NaN3
4. Determine staining volume for all containers (i.e. 2 mL for each single well of a 6-well plate).
5. For every 1 mL staining volume required, add 1 mL of 0.1% Triton-X 100 containing 1 ug/mL Tx-Red-Maleimide and 1.5 ug/mL DAPI.
6. Let sit on a rocker for at least 2 hours. Remove stain solution.
7. Quench reaction by add 1 mL of 3% BSA in PBS and let rock for 15 min.
8. Rinse with DPBS/NaN3. After last rinse add enough PBS/NaN3 volume to sufficiently cover the cells.

10a. If stored for a long period of time, make sure container is sealed so water does not evaporate from the dish.

Optional: You can add 1 ug/mL DAPI to the storage solution and you can add glycerol (i.e. 50%) to the storage solution. We do not generally use glycerol because it appears to reduce the quality of the phase imaging of the cells and fibrils.

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General cell seeding procedures for cell morphology measurement protocol

Jte
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Summary: This is the procedure typically used for generating cell morphology data of low density cells.

1. Culture cells as normal. Cell seeding densities used for experiments are between 800-1200 cells/cm2 for larger A10-like and smaller NIH3T3-like cells, respectively.
2. Prepare test substrates (i.e. such as collagen). Preincubate the cells in complete media to equilibrate if required.
3. Prepare cells by trypsinization. Count and collect cell volume data. Record filename. Use phase microscopy to ensure you have a single cell suspension.
4. Seed cells at appropriate density (~1000 cells/cm2). Attempt to ensure the seeding density is relatively homogeneous across the surface.
5. Place cells at 37C for desired time (i.e.16 hours).
6. Fix cells in 1-4%PFA in PBS if only morphology is being performed. Morphology appears to be well preserved under these conditions.
7. Stain with TxRed and DAPI (see protocol).
8. Collect images with automated microscopy and analyze with ICA software.

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PWFCE_2ColorMorphologyGeneral_0001.png

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Fixing cells in 1% formaldehyde for 1h in PBS.

Jte
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Summary: This is a general 1% PFA in PBS fixing procedure for cell cultures in plates.

1. Open 16%FA vial (EMS). Use several layers of parafilm to reseal. Can be stored in refrigerator for several months after opening.
2. Make a 4%(v/v) formaldehyde solution with PBS. For 10 mL of PBS, add 2.5 mL of 16% PFA
3. Remove media from cells by aspiration.
4. Rinse with complete media if required. **This step is not required in most cases. PBS can be used for rinsing, but we have evidence that suggests PBS can cause cells to deattach or smear on the culture dish. PBS rinses works best in large surface area containers.**
5. Gently add fixative to cells. Enough fixative to clearly cover the cells should be used.
6. Incubate at room temperature for at least 1h.
7. Remove fixative with aspiration, rinse with PBS/N3.
8. Proceed with staining steps or keep fixed cells in PBS/N3 at 4C until use.

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PXXCE_1PercentParaformaldehydeFixation_0003.png

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SUBCULTURE OF NIH3T3 (ATCC, CRL-1658)

10/2/2008

REAGENTS REQUIRED:

DPBS, Ca and Mg free (Gibco or Sigma)
0.25% trypsin-EDTA, 1x, (Sigma)
growth medium

PROCEDURE

1. Warm-up all reagents at 37 C. Avoid warming the whole bottle of medium: instead dispense 6-7 or 10-11 ml of medium in a T-25 or 10-cm dish, respectively and place in incubator to equilibrate
2. Remove and discard spent medium
3. Add 3 –5 ml (depending on flask size) of DPBS into flask. Avoid pipetting fluid directly on cells. Gently rock the flask few times, then discard the DPBS.
4. Add enough trypsin to cover the cell layer (usually, 0.5 ml/25-cm^2 flask, 2 ml/150-cm^2)
5. Incubate at 37 C until most cells have detached (usually about 3'): tap gently bottom of flask to help dislodging cells.
6. Stop trypsin with 2x volume of serum-containing medium or, if growing cells in serum-free medium, of trypsin inhibitor solution. Resuspend well. Break clumps, if present, by resuspending few times with a Pasteur pipette.
7. For routine subculture it is not necessary to spin down the cells; otherwise, spin cells at 100 g for 5'. Discard supernatant and resuspend in 1 ml of medium from the flask/dish previously placed in the incubator.
8. Count cells with Coulter counter and save file as "NIH3T3 p.xx mm/dd/yy", where p.xx is the passage number
9. Seed cells at 3000/cm2 (for a 3-day spit) or 1700/cm2 (for a 4-day split. After returning the flask to the incubator, slide it on the surface in a cross-like pattern to evenly distribute the cells.

NOTE: it is common for these cells to undergo a post-thaw crisis. After the first subculture following thawing, cells stop growing, and most of them are poorly spread. This lasts for few days: in most cases, cells will then recover and resume normal proliferation and morphology.

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PXXCE_NIH3T3CellMaintenance_0003.png

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Use of Falcon-6-well TCPS as a cell culture substrate

Jte
9/12/08

Summary: New tissue culture polystyrene plates and dishes are generally supplied by Falcon, but Corning and others have been used.

1. Cells are seeded in 6-well Falcon plates (353046) TC treated. Cells are directly added to well. No preconditioning is used.

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PXXCE_TCPS6WellPlate_0003.png