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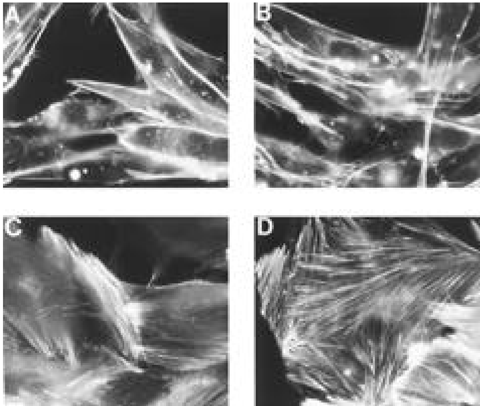
Stress Fiber Protocol

DragonTech
A Biotechnology Services Company

Introduction: Stress fibers are a specific cytoskeletal organization of actin monomers. These fibers are involved in cell shape a structural functions of the cell. Actin monomers form long polymers, which attach to the plasma membrane at focal adhesions. Contraction of the actin stress fibers allows the cell to exert tension on the substratum, an important part of controlling morphogenesis. Formation of stress fibers and focal adhesion complexes are a key regulatory event in cell growth and cell movement such as migration and invasion.



When conducting the experiments it is crucial that you thing about what you are planning to do! Not just the technical aspects of the experiments but what KIND of data are you going to get. If you are just looking for a picture of stress fibers vs actually counting the number of cells that are forming the fibers it will be very important spend the time getting the right kind of pictures. Just getting a picture does you no good if you also need to determine the percent of cells that are actually displaying strong organized fibers. Take several picts for each slide/condition. Make certain they are centered in the screen. Use the example to the left to make that decision. When counting you need to count several fields from each slide. For EACH field, count 1) the total number of cells; 2) the number of cells that display stress fibers and if necessary, 3) the number of cells that are showing weaker formation. The figure above has a control in A., week formation in C and stronger stress fiber formation in D. Look at the following papers for examples : Kam and Exton, Mol Cell Biol. 2001 Jun; 21(12): 4055-66, and Tominaga T, Ishizaki T, Narumiya S, Barber DL. EMBO J. 1998 Aug 17; 17(16): 4712-22.

Coverslip Preparation:

1. Determine the number of experiments needed and *make certain there are enough sterile coverslips in advance.*
2. For each experiment place one sterilized coverslip into a sterile 35 mm culture dish.
3. Pipette 1.5 mL of complete DMEM into each dish and incubate for 20 minutes at room temperature in the hood.
4. Remove the media and allow the coverslips to air dry in a closed hood for at least 10 minutes.

Cell Preparation: (*the cells should be very sparse after splitting. If they are more than 50% confluent at the time of starving the experiment will not work*).

1. Trypsinize 1 T25 flask of cells and add 9 mL of complete DMEM media
2. Using a hemocytometer, count cells and calculate cells per ml.
3. Seed 35 mm dishes with approximately 30,000 – 50,000 cells in 2 ml of complete media.
   a. Alternatively (should seed by counting) splitting a cell culture from a 80% confluent T-25 flask into 18 dishes should be close to the correct final seeding density.
4. Place cells in incubator over night.
5. Remove complete media and gently rinse with sterile 2 ml CMF-PBS.
6. Add 2 mL of Spinner Media (0.5% serum)
7. Place cell in incubator overnight.

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Starving Cells:

1. Remove spinner media and gently rinse with 2 ml CMF-PBS
2. Add 2 mL of DMEM starving media
3. Place cells in incubator for 1 hour.

Fixing and Permeablizing Cells:

1. Following appropriate cell treatments gently remove media.
2. Rinse with chilled CMF-PBS 3 times.
3. Gently pipette 1.5 mL of chilled 0.5% paraformaldehyde into each dish.
   NOTE: Make Paraformaldehyde Fresh Daily
4. Incubate dishes in refrigerator for 15 minutes.
5. Remove paraformaldehyde
6. Gently rinse 3 times with chilled CMF- PBS.
7. Gently pipette with 1.5 mL of chilled 0.1% Triton X-100 into each dish
8. Incubate in refrigerator for 10 minutes.
9. Gently rinse with CMF-PBS 3 times.

Phalloidin Staining Cells:

1. Pipette 1 mL of CMF-PBS per dish into 15 mL falcon tube
2. Add 1μL of phalloidin stock solution for each 1 mL of CMF-PBS.
3. Pipette 1 mL of phalloidin solution into each dish making sure to cover the coversslip completely.
4. Incubate at room temperature for 40 minutes.
5. Remove the solution and rinse with CMF-PBS 3 times for 5 minutes each.

Adhering to slides:

1. Prepare Prolong antifade reagent. (*found in freezer – make sure you put it back*)
   - Add 1 ml (32 drops) Prolong mounting medium to one of the brown vials containing Prolong antifade reagent.
   - Mix by gently pipetting up and down until antifade reagent no longer adheres to sides of brown vials.
   - Continue mixing by pipetting or vortexing.
   - Remove any bubbles formed during mixing by sonication.
2. Remove final rinse of CMF-PBS.
3. Using a tweezers pick up the cover slip and blot edges on a Kimwipe to remove excess liquid.
4. When slide is nearly dry, add one small drop of Prolong Antifade Mixture to the cell side of the coverslip.
5. Place coverslip cell side down on a clean microscope slide.
6. Place slide with the mounted coverslip into a dark place to dry.
   NOTE: slides must be laid flat and could take 1 – 2 hours to dry. The more Prolong Antifade mixture used the longer it will take to dry.
7. Once antifade mixture is dry, seal coverslip to slide with fingernail polish.

Recipes

Phalloidin (*in freezer, in metal can*)

Stock: 0.1mg of Phalloidin powder
1 mL of DMSO
Put in small microfuge tubes in 10 µL aliquots

Staining: Add 1 µL of Phalloidin stock 1 mL of CMF-PBS
(mix in falcon tube and put on slips)

0.1% Triton-X 100 (40 mL)
200 µL 20% Triton-X 100
40 mL CMF-PBS
mix in large falcon tube and refrigerate

0.5% Triton-X 100 (40 mL)
1.0 mL 20% Triton-X 100
40 mL CMF-PBS
mix in large falcon tube and refrigerate

10% Paraformaldehyde Solution
1 g Paraformaldehyd
10 ml water
Mix vigorously (If not completely dissolved)
1-2 drops 1 M NaOH
Heat to 65°C
Mix Vigorously

0.5% Paraformaldehyde-
1 ml 10% paraformaldehyde
19 mL CMF-PBS
Mix Vigorously