FLUORESCENT STAINING OF CELLS

Fluorescent Phalloidin Staining

Materials

1. Fluorescent phalloidin in methanol. Phallacidin does not work as well. Dilute 10 ul 330 nM stock into 500 ul PBS for each large coverslip.

2. PBS, solution A.

Procedure

1. Fix and permeabilize cells (see other protocols). Mount coverslip onto a plastic frame reserved for fixed samples.

2. Turn off light. Dilute fluorescent phalloidin 50x into PBS.

3. Gently pipet phalloidin solution onto coverslip. Stain for 30 min at room temperature.

4. Rinse coverslip 3x with PBS.

5. Fill the chamber with PBS or an antibleaching solution and observe. Dishes may be stored at 4^{o}C in a sealed, light-tight container.

Immunofluorescence Staining

Materials

1. PBS/BSA: PBS solution A with 1% BSA (Boehringer Mannheim 100 350) and 0.1% NaN_3, stored at 4^{o}C. Bring to room temperature before use.

2. Primary antibody, diluted appropriately with PBS/BSA. Need 200 ul per 45x50 mm coverslip. Clarify in a Eppendorf for 15 min (minimal requirement) or in an ultracentrifuge with the Type 42.2Ti rotor (or Airfuge) if necessary.

3. Secondary antibody, prepared as for the primary antibody.

4. Coverslip boxes/containers.

Procedure (do not allow coverslips to dry out anytime)

1. Fix and permeabilize cells (see other protocols). Wash with PBS/BSA for 10 min in a fixation box.

2. Cut a small piece of parafilm to match the area of staining and put 200 ul antibody solution on the piece. Shake off most of the liquid from the coverslip but do not let it dry out. Invert the coverslip onto the parafilm. Prepare a 100 mm plastic petri dish containing a piece of wet filter paper. Place 2 wooden sticks in the dish and put coverslip upside down on the sticks. Seal the dish into a ziplock bag and place in the incubator. Stain 45 min at 34-37^{o}C with the primary antibody, or overnight at 4^{o}C.

3. Wash gently 3x, 10 min each, with PBS/BSA on a shaker. Fill a coverslip box with PBS/BSA and sink the coverslip to the bottom. The covering parafilm should float up.

4. Stain 30 min with the secondary antibody as in step 2.

5. Wash as in step 3.

6. Mount the coverslip onto a plastic frame reserved for fixed coverslips. Fill the chamber with PBS or an antibleaching solution and observe. Dishes may be stored at 4^{o} in a sealed, light -tight container.
