FIXATION WITH FORMALDEHYDE-ACETONE

Materials

1. Dulbecco's PBS, 37^{o}C.

2. Fixation buffer, i.e. PBS, PHEM, or modified Hanks ("cytoskeleton buffer"), 1.33x.

3. Formaldehyde, 16%, EM Sciences. Store at room temperature in the fume hood. Mark the date when you open a new ampule. Seal tightly with parafilm. Handle with caution.

4. Fresh 4% formaldehyde solution. Mix 3 parts 1.33x fixation buffer with 1 part 16% formaldehyde (e.g. 9 ml and 3 ml for 1 coverslip), in a bottle designated for fixatives. Cap tightly and warm up in a water bath.

5. Acetone, chilled to -20^{o}C in a freezer. Transfer into 100 ml beakers designated for fixation, each coverslip needs a separate beaker.

6. Forceps for fixation.

7. Plastic fixation boxes.

Procedure

1. Transfer the warm fixation solution into a plastic fixation box immediately before fixation.

2. Drain all the medium from the coverslip dish. Rinse out 2 times with warm PBS.

3. Quickly and carefully remove the coverslip.

4. Place on the surface of the fixation solution, cell side facing down. The coverslip should float on the surface of the solution. Rock the box intermittently. Fix for 10 min at room temperature.

5. Remove the coverslip with a pair of fixation forceps. Rinse with distilled PBS 2 times.

6. Dip the coverslip into the cold acetone. Rock gently. Let sit for 5 min.

7. Remove the coverslip from acetone and rinse immediately with PBS. Do not let the surface dry out.

8. Proceed with staining.

9. Used acetone should be emptied into a designated container.

10. Note: The acetone step may be replaced by adding 0.1% Triton X-100 to solution 4 and do fixation and extraction in one step.