Actin and Nuclei Staining of Mammalian Cells

1. Place cells on chambered slide or coverslip and allow to attach for 30 minutes.

2. Remove media and immediately wash cells twice with prewarmed phosphate-buffered saline, pH 7.4 (PBS).

3. Fix the sample with freshly made 3.7% formaldehyde solution in PBS for 10 minutes at room temperature. (If using chambered slides, remove wells after incubation)

4. Wash two times with PBS.

5. Add 0.1% Triton X-100 in PBS for 3 to 5 minutes.

6. Wash two times with PBS.

7. Place the TRITC-phalloidin solution on the slide for 20 minutes at room temperature. To avoid evaporation and photobleaching, keep the coverslips inside a covered container, in the dark during the incubation.

8. Wash two times with PBS.

9. Add 50 μl of DAPI to each well. Let sit 5 minutes.

10. Gently wash off DAPI with water.

11. Add a couple of drops of mounting solution and apply coverslip.

12. Seal edge of slide with nail polish.

TRITC-Phalloidin solution (make fresh):

40 μl TRITC-phalloidin stock
960 μl PBS