Phalloidin stain for formaldehyde-fixed cells (Texas Red-X phalloidin, Invitrogen T7471)

1. Wash cells twice with prewarmed phosphate-buffered saline, pH 7.4 (PBS).

2. Fix the sample in 3.7% formaldehyde solution in PBS for 10 minutes at room temperature.

Note: Methanol can disrupt actin during the fixation process. Therefore, it is best to avoid any methanol containing fixatives. The preferred fixative is methanol-free formaldehyde.

3. Wash two or more times with PBS.

4. Place each coverslip in a glass petri dish and extract it with a solution of acetone at ≤–20°C or 0.1% Triton X-100 in PBS for 3 to 5 minutes.

5. Wash two or more times with PBS.

6. When staining with any of the fluorescent phallotoxins, dilute 5 µL methanolic stock solution into 200 µL PBS for each cover­slip to be stained. To reduce nonspecific background staining with these conjugates, add 1% bovine serum albumin (BSA) to the staining solution.

7. Place the staining solution on the coverslip for 20 minutes at room temperature (generally, any temperature between 4°C and 37°C is suitable). To avoid evaporation, keep the coverslips inside a covered container during the incubation.

8. Wash two or more times with PBS.

9. For long-term storage, the cells should be air dried and then mounted in a permanent mountant such as ProLong® Gold reagent or Cytoseal. Speci­mens prepared in this manner retain actin staining for at least six months when stored in the dark at 2–6°C.