

# BIOIMAGING PLATFORM

## Single and multicolor imaging of fixed specimen: fixation protocols

The quality of immunofluorescence of cytoplasmic antigens depends very much upon the right choice of a fixation protocol. We are suggesting two different protocols that work well for most antigens.

### A. Paraformaldehyde fixation

Crosslinks proteins and nucleic acids by forming methylene bridges between reactive groups.

#### **Standard paraformaldehyde fixation protocol for cell cultures growing on glass coverslips**

1. Fix specimen with 3% paraformaldehyde in PBS for 20 min at RT.
2. Wash 3 x 5 min with PBS.
3. Incubate 5 min with 20 mM glycine in PBS.
4. Permeabilize with 0.1% Triton X-100 in PBS for 5 min.
5. Wash 2 x 5 min with PBS.
6. Place specimen in humidified chamber and incubate with appropriate primary antibodies for 20-60 min.
7. Wash 4 x 5 min with PBS.
8. Place specimen in humidified chamber and incubate with appropriate secondary flourochrome-conjugated antibodies for 20-60 min.
9. Wash 4 x 5 min with PBS.
10. Dry off excess PBS and mount specimen on glass slides with DABCO (1,4-Diazabicyclo(2.2.2)octane) antifading solution (PBS containing 50% glycerol and 2% DABCO).
11. Seal coverslips with nail polish.

### B. Coagulating fixation - Methanol and acetone

- Fix specimens by rapidly changing hydration state of cellular components
- Proteins are either coagulated or extracted
- Preserve antigen recognition often
- But cause significant shrinkage of specimen

#### **Standard methanol-acetone fixation protocol for cell cultures growing on glass coverslips**

1. Grow cells on glass coverslips.
2. Remove coverslips from dish, dry off excess medium and put for 5 min into methanol at  $-20^{\circ}\text{C}$ .
3. Remove coverslips and immediately dip in acetone at  $-20^{\circ}\text{C}$ . Leave for 5 min and then transfer into PBS.
4. Continue with step 6 of paraformaldehyde fixation protocol.