Fixation & staining for fluorescence microscopy

→ for fixation see Standard Protocol for Fixation and Staining

- RT / 10 min
- wash 2x PBS + 0.1 M ammoniumchloride
- wash with PBS
- permeabilise with PBS + 0.2% triton X-100 (stock: 2%)
- RT / 5min
- wash 3x PBS
- block with 0.1% fish skin gelatine in PBS (stock: 1%)
- 4°C / overnight
- 1st antibody in adequate dilution in staining buffer (PBS + 0.1% gelatine + 1% FCS)
- RT / 3 h
- wash 3x PBS
- 2nd antibody in staining buffer (+ DAPI: 1:2000 if required)
- RT / 30 min
- wash 5x PBS
- dip in H_2O, let dry almost completely
- mount in adequate mounting medium.

→ If ProLong Mounting Medium (Invitrogen P36930) is used, let samples dry for several days, then they can be stored at -20°C for a year or so. But ProLong is not compatible with Q-Dots!

→ mounting medium with DAPI for nuclear stain can be used, but including DAPI in the staining buffer gives less background staining