Preparation of samples for light/confocal microscopy

Source: Multi-parametric bioimaging and cytometry core facility of the OVGU

Note: Different specimens may require different preparation methods. Therefore, the testing and optimizing of each step within the protocol for each new sample type will ensure that the best balance between preservation and labeling is obtained.

Step	Temperature	Duration
Fixation 4 %PFA/0.02 %GA in PBS (pH 7.4)	RT	10 min
wash 3x in PBS	RT	5 min
Permeabilization 0.2 % TX100 in PBS	RT	5 min
wash 3x in PBS	RT	5 min
Blocking-step 1 % BSA in PBS	RT	10 min
Primary antibody incubation	RT	1 h
wash 3x in PBS	RT	5 min
Block 1 % BSA in PBS	RT	10 min
Secondary antibody incubation	RT	1 h
wash 3x in PBS	RT	5 min
Postfixation 4 % PFA/0.05 % GA in PBS	RT	10 min
wash 3x PBS	RT	5 min
wash 1x PBS (pH 8.9)	RT	5 min

This protocol was optimized for fibroblast cells:

Embed in mounting medium:

Vectashield (Vector Labs) + glycerol 1:1 and 2 % DABCO

Sealing the samples:

Nail polish or nail hardener or VALAP.

VALAP is a 1:1:1 mixture of vaseline, lanolin and paraffin. It is prepared by mixing the components in a bottle on a heating plate. The mixture is applied with the help of a thin tip and hardens immediately. It can be reheated and reused many times.