Labeling of mammalian living cells with live-cell probes

Materials

Forceps
For handling cover slips, forceps with very fine tips are recommended, e.g. Dumont forceps No. 5 (straight) or Dumont forceps No. 7 (bent).

Imaging medium
For staining procedure, washing and mounting cells, growth medium (depending on the application, cell line etc.) without pH indicator as phenol red should be used because the pH indicator may affect the imaging. We highly recommend using growth medium containing live-cell compatible radical scavengers as ascorbic acid. An example of a commercialized imaging medium is DMEMgfp-2 (Evrogen, Moscow, Russia).

Fluorophore-labeled probes for live-cell staining !TOXIC!
Typically probes are shipped freeze-dried (i.e. in a solid form). Upon arrival the conjugate needs to be dissolved in DMSO (water-free) creating a stock solution (for molecular mass of each conjugate please see the leaflet or visit our web shop). Please note, after dissolving the conjugate you need to store the stock solution always at -20°C for longer shelf life. For labeling of mammalian cells, this stock solution needs to be diluted in cell growth medium to a final concentration of ~1 - 2 µM in cell growth medium (depending on the application, cell line etc.).

Fluorescent Dye	Ex_{max}	Em_{max}	Recommended STED Laser
Abberior LIVE 510	498	529	580-600
Abberior LIVE 515	515	541	590-610
Abberior LIVE 580	603	638	750-780

Cover slips / live-cell chamber
For the use of oil and water immersion objectives, glass cover slips with a thickness of ~170 µm should be used i.e. No. 1.5 or No. 1.5H. Alternatively live-cell chambers as chamber slides or glass bottom petri dishes can be used.
We recommend not to use plastic cover clips or live-cell chambers with plastic bottom because frequently only suboptimal imaging results may be achieved.
Further no cover slips with grids, gratings or similar should be used because those structures might interfere with imaging, i.e. aberrations may occur.

Slides
We recommend mounting cover slips (! Cover slips with 18 mm diameter are required !) on slides with a small cavity as they will provide enough medium for live-cell measurements. Limitation for slides is that they need to fit to the sample holder of the microscope. Super frost slides or similar slides are not recommended.

Plastic or glass petri dishes
For washing of the samples…

Labeling of cultivated mammalian living cells

1. **Cultivation of cells**
   The cells are typically seeded on cover slips or in cell culture chambers 12 – 36 h before labeling. Seeding and growth is done in standard growth medium.

2. **Preparing the staining solution**
   The staining solution is prepared by dissolving parts of the stock solution of the probe in imaging medium to a final concentration of 2 µM.
   ! Please note that the staining solution is not stable for extended time. Prepare only the amount that is used immediately !

3. **Labeling**
   Before labeling, the cells are washed once in imaging medium.
   Then the cells on cover slips or in cell culture chambers are incubated for 1 h at cell growth conditions (CO_2-buffered environment) in the staining solution (2 µM).

4. **Washing**
   Remove the staining solution.
   After removing the staining solution, cells are washed once with fresh imaging medium.

5. **Embedding**
   a. **Cover slips**
      Finally cover slips are being taken out of the petri dish used for washing; excess liquid is removed by placing the cover slip with the edge on a piece of tissue. Then the cover slips are mounted onto a slide with cavity (cells facing the slide) which is filled with fresh imaging medium (90 µl). Excess imaging medium is removed using tissue. Pressing the cover slip firmly on the object slide will prevent the cover slip from moving off the object slide.
   b. **Live-cell chambers**
      After washing, the medium in the live-cell chambers is exchanged against fresh imaging medium.

6. **Storage**
   Ready-made samples should be used immediately. To keep the cells alive for a longer time, place them in a cell incubator or use a live-cell incubation chamber for the microscope while doing the microscopy experiment.