

Immunofluorescence with LEC

Protocol for Lab-Tek II Chamber Sildes (Nunc, 154535)

Material

- Lab-Tek II Chamber Sildes, 8 chambers (Nunc, 154535) coated with poly-D Lysin in PBS ($5\mu\text{g}/\text{cm}^2$)
- PBS⁺⁺
- 4% PFA in PBS
- 0.1% Triton/BSA in PBS
- 1% BSA in PBS⁺⁺
- DAPI/PBS ($1\mu\text{g}/\text{ml}$)
- ProLong Antifade Kit
- LEC, 1 isolation Fl. 1 P6

Antibodies (for example!)

- anti-human LYVE-1, Rabbit, use 1:100 (RELIATech Cat# 102-PA50)
- anti-human Prox-1, Rabbit, use 1:100 (RELIATech Cat# 102-PA32)
- anti-human CD31, Mouse, use 1:100 (DIANOVA Cat# 10338)

Protocol

- wash cells 2x with PBS
- fix cells for 5min at RT with 4% PFA in PBS
- wash cells 2x with PBS
- incubate for 15min in 1% BSA/PBS at RT
- perforation of membrane by incubation in 0.1% Triton/BSA
- wash cells 2x with PBS
- incubation with primary antibody for 1h at RT
- wash cells 2x with PBS
- incubation with secondary antibody for 45min at RT
- wash cells 2x with PBS
- incubation for 10min at RT in DAPI
- cover cells with the ProLong Antifade Kit

☞ samples are ready for analysis with the microscope!