Double staining of lymphatic endothelial cells and HUVECs
(Protocol for cell culture dish)

Material
- PBS^{++}
- 4% PFA in PBS
- 0.1% Triton/BSA in PBS
- 1% BSA in PBS^{++-}
- DAPI/PBS (1µg/ml)

Antibodies (for example!)
- anti-human Prox-1, Rabbit, use 1:1000 (RELIATech Cat# 102-PA32)
- anti-human CD31

Protocol
- wash cells 2x with PBS
- fix cells for 5min at RT with 4% PFA in PBS
- wash cells 2x with PBS
- incubate for 45min in 1%BSA/PBS at RT
- perforation of membrane by incubation in 0.1% Triton/BSA for 5min
- wash cells 2x with PBS
- incubation with primary antibody for 2h at RT
- wash cells 2x with PBS
- incubation with secondary antibody for 1h at RT
- wash cells 2x with PBS
- incubation for 10min at RT in DAPI (optional)
- cover cells with Flouromount directly in the culture dish

✍ samples are ready for analysis with the microscope!