FACS analysis with human macro-/microvaskular endothelial cells

- 50.000 to 100.000 cells/well (e.g. HUVEC, HDMVEC)

Protocol:

- wash cells with PBS (2ml/T25)
- add 1.5 ml Accutase/T25 and incubate for 2-15 min.
- detach cells by knocking the flask
- rinse the flask with PBS/2% FCS and transfer cell solution in a 15 ml tube
- 1000 rpm, 5 min.
- wash cell pellet with 5 ml PBS/FCS
- 1000 rpm, 5 min.
- resuspend the cells with PBS/FCS to the appropriate density and transfer 200 µl/well into a microtiter plate (with round botton)

Antibodies:

1. anti-LYVE-1 (**RT #102-PA50**); [c = 1000], rabbit polyAB; 1:1000
   as 2. AB: anti-rabbit FITC (Dianova);

2. anti-FLT-4 (mabc=860) mouse mab, 1:400
   as 2. AB: anti-mouse PE, 1:300, 0.8 µl/well, Jackson Immuno Research 115-116-146

3. anti-Podoplanin, (**RT #101-M40**) biotinyl. mouse mab, [c = 1380]; 1:500;
   as 2. AB: Strep-APC, Pharmingen 554067, 1:2000

4: anti-KDR (**RT #101-M20**); mouse mab, [c=5000]; 1:1000
   as 2. AB: anti-mouse FITC (Dianova)

Controls: - cells without primary antibody
- only secundary antibody

Note: The optimal dilution must be determined for each antibody!.

- incubate 30 min in the dark on ice, resuspend the cells after 15 min
- 10 min, 1200 rpm
- empty the plate carefully (1x)
- wash cell pellet in 200 µl FACS buffer
- 10 min, 1200 rpm
- empty the plate carefully (1x)
- add 2. antibody in 100 µl FACS buffer
- incubate 30 min in the dark on ice, mix once

- 10 min, 12000 rpm,
- wash the cell pellet like described above
- add 400 µl FACS buffer containing Propidiumiodid into a FACS reaction tube
  (PI= 0.5 µl to 500 µl total solution)
- resuspend cells in 100 µl FACS buffer, mix with 400 µl and keep in the dark on ice
- measurement