Immunofluorescence staining protocol: CD13

Version: August 2021

Evercyte Protocol No.:	IF_{FC}-CD13-V3
Cells:	E.g. RPTEC/TERT1 (Evercyte, Cat# CHT-003-0002)
Reagents:	0.05% Trypsin-EDTA (Gibco, Cat# 25300-054, ready-to-use, stored at 4°C after thawing) Defined Trypsin Inhibitor (Gibco, Cat# R007100, ready-to-use, stored at 4°C after thawing) PBS (Sigma-Aldrich, Cat# D8537, ready-to-use, stored at 4°C) 10 % FBS in PBS (=blocking solution, Sigma-Aldrich, Cat# F7524, ready-to-use, stored at 4°C) DAPI (Roche, Cat# 10236276001, stored in aliquots at -20°C; diluted 1:50 in PBS) Anti-Human CD13 (mouse, SouthernBiotech, Cat# 9556-01, stored at 4°C; diluted 1:100 in blocking solution) Anti-mouse IGG (H+L), Alexa Fluor® 488 (donkey, Jackson ImmunoResearch, Cat# 715-545-150, stored in aliquots at -20°C; diluted 1:500 in blocking solution)
Staining procedure:	- detach the cells from the culture vessel by using PBS and Trypsin-EDTA as described in *Protocol for Passaging of RPTEC/TERT1* and determine the viable cell number - transfer about 5 x 10^5 cells to a 15 ml centrifugation tubes (for each test approach) - centrifuge at 170 g for 5 min - discard the supernatant - resuspend the resulting cell pellet in the remaining droplet - add 200 µl blocking solution to each test sample and incubate for 15 min at 37°C - add 2 µl of undiluted primary antibody (final dilution is 1:100 in blocking solution) and incubate for 30 min at 37°C (cells incubated with an isotype control antibody are used as control) - add 10 ml of PBS and centrifuge at 170 g for 5 min - discard the supernatant - resuspend the resulting cell pellet in the remaining droplet - add 500 µl secondary antibody dilution (1:500 in blocking solution) and incubate at 37°C for 30 min - add 10 ml of PBS to each tube and centrifuge at 170 g for 5 min - discard the supernatant - resuspend the resulting cell pellet in the remaining droplet - add 400 µl DAPI dilution (1:50 in PBS) - analyse for CD13 expression immediately by flow cytometry (Alexa Fluor® 488: excitation 499 nm, emission 520 nm; DAPI: excitation 359 nm, emission 457 nm)

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