Immunofluorescence staining protocol: Vimentin

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Evercyte Protocol No.:	IF_M-Vimentin-V2
Cells:	E.g. fHDF/TERT166, HDF/TERT164, primary fibroblasts from different tissues
Reagents:	PBS (Sigma-Aldrich, Cat# D8537, ready-to-use, stored at 4°C) 4 % PFA / Histofix (RothiLab, Cat# P087.4 ready-to-use, stored at RT) DAPI (Roche, Cat# 10236276001, stored in aliquots at -20°C; diluted 1:50 in PBS) (1 ml PBS + 20 µl DAPI) 0.3 % Triton-X-100, 10 % FBS in PBS (= blocking solution) - 9 ml PBS - 30 µl Triton-X-100 (Sigma, Cat# T8787, ready-to-use, stored at RT) - add 1 ml FBS (PAN Biotech, Cat# P30-3031, ready-to-use, stored at 4°C) - mix properly - prepare freshly before use Anti-Human Vimentin-AF® 488 (mouse, BD Pharmingen, Cat# 562338, stored at 4°C; diluted 1:20 in blocking solution (142.5 µl blocking solution + 7.5 µl AB) IgG1 k isotype control antibody-AF® 488 (mouse, BD Pharmingen, Cat# 557721, stored at 4°C; diluted 1:20 in blocking solution) (142.5 µl blocking solution + 7.5 µl AB)
Staining procedure:	- detach the cells from the culture vessel by using PBS and Trypsin-EDTA as described in the respective *Protocol for Passaging of Cells* and seed the cells into IBIDI slides (24-72 hours before staining), cells should have reached about 60-90% confluence before staining - discard the supernatant and wash the cells twice with PBS (about 200 µl each) - add about 200 µl fixation solution and Incubate at RT for 10 min - wash the cells 3 times with PBS (about 200 µl each) - add 150 µl vimentin antibody and incubate at RT for 60 min (light protected) - wash the cells 3 times with PBS (about 200 µl each) - add about 200 µl DAPI solution and incubate at RT for 10 min - wash cells once with PBS and add 150 µl PBS / well - analyse the samples using fluorescence microscopy (Alexa Fluor® 488: excitation 488, emission 520 nm; DAPI: excitation 359 nm, emission 457 nm) - interpretation: typical staining of cytoskeletal fibers

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