Immunofluorescence staining protocol: ZO1

Version: August 2021

Evercyte Protocol No.:	IF_M-ZO1-V2
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 RT) PBS^{++} with $MgCl_2$ and $CaCl_2$ (Sigma-Aldrich, Cat# D8662, ready-to-use, stored at 4°C) ROTI®Histofix 4 % (Formaldehyde 4 %, Carl Roth, Cat# P087.4, ready-to-use, stored at RT) 5 % donkey serum in PBS^{++} (=blocking solution, Jackson ImmunoResearch, Cat# 017-000121, diluted with PBS^{++}, stored in aliquots at -20°C) Triton-X-100 (Sigma-Aldrich, Cat# T8787, stored at RT) DAPI (Roche, Cat# 10236276001, stored in aliquots at -20°C; diluted 1:50 in PBS) Anti-human ZO1, primary antibody (rabbit, Thermo Fisher, Cat# 40-2200, stored in aliquots at -20°C, diluted 1:100 in blocking solution) Anti-rabbit IgG (H+L), Alexa Fluor® 594, secondary antibody (donkey, Jackson ImmunoResearch, Cat# 711-585-152, stored in aliquots at -20°C, diluted 1:1000 in blocking solution)
Fixation and permeabilization:	- remove the supernatant and wash the cells (confluent monolayer!) twice with PBS - add ROTI®Histofix 4 % and incubate for 10 min at RT - remove ROTI®Histofix 4 % and wash the cells 3 times with PBS - use fixed cells immediately for staining, do not store for prolonged period of time - add blocking solution containing 0.3 % Triton-X-100 and incubate for 20 min at RT
Staining procedure:	- add primary antibody dilution (1:100 in blocking solution) and incubate for 60 min at RT (cells incubated with an isotype control antibody are used as control) - wash the cells 3 times with PBS - add secondary antibody dilution (1:1000 in blocking solution) and incubate for 60 min at RT - wash the cells 3 times with PBS - add DAPI dilution (1:50 in PBS) and incubate for 15 min at RT - wash the cells 3 times with PBS and remove washing solution - add PBS to cover the cells and keep the slides at 4°C until analysis is performed using fluorescence microscopy (Alexa Fluor® 594: excitation 590 nm, emission 618 nm; DAPI: excitation 359 nm, emission 457 nm)
Interpretation of data	- ZO1 is localized at cell membranes at sites of cell-cell-contacts

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