[Protocol 4.05] IF staining PluriQC

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Version: 1.0

Media and Reagents:
Anti-SSEA4-PerCpVio700 (Miltenyi Biotec, 130-105-053)
Anti-Oct3/4-APC (Miltenyi Biotec, 130-105-555)
Anti-Tra1-60-Vio488 (Miltenyi Biotec, 130-106-872)
Anti-Nanog -PE (Cell Signaling, 14955)
DAPI/ Hoechst (Thermo Fisher, 62249)
Essential 8 Media Kit (Thermo Fisher, A1517001)
Pen/Strep (Thermo Fisher, 15140-122)
Geltrex (Thermo Fisher, A1413302)
KO DMEM/F-12 (Thermo Fisher, 12660-012)
PBS with!!! Ca2+ and Mg2+ (Thermo Fisher, 14040-091)
Roti®-Histofix 4 % (Roth, P087.4)
Saponin (Sigma, 47036)
MACS® BSA Stock Solution (Milteny Biotec, 130-091-376)

Materials and Equipment:
Geltrex coated coverslips in 12 well plates
Aspiration pump
5ml/10ml pipettes
15ml conical tube

1. Seeding cells
1. Prepare Geltrex coating of coverslips in 12 well plates. Prepare at least three wells for staining.
2. Prepare single cell suspension of hiPSCs from one 6-well with (60 -70% dense) regarding to Protocol 1.03.0 Passaging of iPSC into single cells using TrypLE/Accutase.
3. Aspirate coating from coverslips in 12 well plates and seed approx. 10.000-15.000 cells in 100µl cell suspension per well.
Note: NHP iPSCs may need different seeding denstiy.
4. Move the plate side to side and back and forward to guarantee an equal cell distribution throughout the wells.
5. Incubate plate at 37°C and 5% CO2 and perform daily medium change (200µl E8 medium per well) until cells are confluent or build adequate sized colonies (usually 2-4 days).

2. IF staining of cells
1. Aspirate media from the cells and wash cells two times with 500 µl PBS with Mg2+ and Ca2+.
2. Fix cells by adding 250µl Histofix solution (4.2 % formaldehyde) for 15 min at RT = stop point
3. Prepare a permeabilisation/blocking buffer (1% BSA; in PBS with Mg2+ and Ca2+) depends on secondary AB
4. Wash cells two times with 500µl PBS with Mg2+ and Ca2+.
5. Permeabilise/block cells with 250 µl buffer for 1 h at RT (shaking is optional).
Note: This is an alternative stopping point. Wash cells two times with 500µl PBS with Mg2+ and Ca2+. Keep the cells in PBS at 4°C for storage up to -4 weeks or perform further staining!
6. Prepare 250 µl of antibody solution in permeabilisation/blocking buffer for each well to be stained. Refer to table 1 for dilution ratios.

Table 1: Recommendation of antibody dilution for IF staining

Antibodies	Dilution antibody
Anti-SSEA4-PerCpVio700	1:100
Anti-Oct3/4-APC	1:100
Anti-Nanog-PE	1:20
Anti-Tra1-60-Vio488	1:100

7. Add 250 µl primary ABs into each well and incubate at 4 degree o.n in the dark (4 degree room = cool room on the shaker).
8. Remove ABs and wash cells three times with PBS with Mg2+ and Ca2+ permeabilisation/blocking buffer. After pipetting PBS (+/+) put on the shaker for 5 min and repeat 2 times = in total three times
9. Add 250 uL of secondary AB + DAPI solution (2 drops in 1 ml) for 1 h at RT in the dark. Dilute with PBS (+/+)
10. Removed secondary AB +DAPI and wash cells three times with 500µl PBS with Mg2+ and Ca2+. Washing step on the shaker for 5 min.
11. Keep the cells in 500µl PBS with Mg2+ and Ca2+ for imaging.
12-. Image the cells immediately or store the completely sealed plate at 4°C in the dark for up to -4 weeks.