Flow Cytometery Protocol (clone 28-8, Ab205921)

Flow Cytometry Materials

- Primary Antibody: Anti-PDL1 (Clone 28-8)
- Isotype Control: Rabbit IgG (ab172730)
- Secondary Antibody: Donkey anti-Rabbit (Fab)'2-APC
- FACS Buffer: 1% BSA / 0.1% NaAzide in PBS (buffer)
- 96 well u-bottom plates, plate sealers, 50mL conical Tubes
- Human Gamma-Globulin
- L2987 (PD-L1 High) and A549 (PD-L1 Low) human tumor cells

Cell Preparation

- L2987 and HT29 cells are harvested using 0.25% Trypsin
- Cells are counted, then centrifuge @ 300G for 5 min
- Wash cells in 10mL buffer
- Bring cells to 10×10^6 cells/mL in buffer

Staining

- Block Fc-receptors using 20µL / mL human gamma-globulin
- Incubate 20 min, on ice
- Transfer 100µL (1×10^6 cells) per well
- Add 1µg / well anti-PDL1 or isotype
- Incubate plate 30 min, on ice, in dark
- Add 100µL buffer, centrifuge 300G for 5 min, decant
- Add 100µL buffer, centrifuge 300G for 5 min, decant
- Dilute Secondary mAb 1:66 (45µL stock + 2.95mL buffer)
- Add 100µL diluted secondary mAb to each well
- Incubate plate 20 min, on ice, in dark
- Add 100µL buffer, centrifuge 300G for 5 min, decant
- Add 100µL buffer, centrifuge 300G for 5 min, decant
- Add 100µL 1% formaldehyde, incubate 20 min on ice in dark
- Add 100µL buffer, centrifuge 300G for 5 min, decant
- Add 200µL buffer per well, seal plate and store in fridge
- Read samples on BD FACS CANTO