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NBP2-29607 Protocol

Assay Instructional Manual (NBP2-29607)

Protocol for immune-phenotype analysis of DC:

1. Wash DC from cultures twice with 1x FACS buffer and suspend in the same buffer.

2. Take typically 0.5-1x10^6 cells in 100ul of buffer for staining.

3. Add 5ul each of CD14 FITC, CD86 AF647, HLA-DR L243 PE, HLA-DR L243PerCP-Cy5.5 and 20ul of CD83 PE antibody, mix well.

4. In alternate tubes, use equal number of cells for unstained and isotype (not provided but details are given) controls.

5. Single color tubes are advised for each analysis.

6. Incubate all tubes on ice for 20 minutes, in the dark, for staining.

7. Wash cells by adding 2ml of 1x FACS buffer, by centrifugation (1200 RPM, 10 min). Repeat twice. Gently aspirate or decant the buffer after each wash and vortex tube at a very low speed, to break the pellet of cells.

8. After final wash, suspend in suitable amount (200-300ul) of staining buffer and analyze the samples.

9. Add PPI as needed to gate live cells for analysis (we typically use 5ul of 50ug/ml stock and incubate cells on ice for a quick/same day analysis).