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NBP2-29607 Protocol

Product Handling Guide (NBP2-29607)

Typical Protocol to generate DC from peripheral blood monocytes:

1. Generate DC following conventional protocol, typically by culturing monocytes with both GMCSF and IL-4, followed by maturation with TNF-alpha.

2. Enrich monocytes from total PBMC by plate adherence method. Briefly, layer PBMC (1-2x10^6/ml) in 6-well plates and allow monocytes to adhere by keeping the culture at 37C for 2h. After 2 hours, remove non-adherent cells. Rinse wells with RPMI -10 complete medium to remove the non-adherent cells completely.
Note: DCD&MM kit works also with DC generated from monocytes enriched by alternate protocols including, magnetic bead separation or RosetteSep.

3. Culture monocytes in 3 ml of complete medium containing recombinant human GMCSF (25 ng/ml) and IL-4 (20 ng/ml). Replenish cultures with fresh medium containing same cytokines.
Note: Concentrations can be standardized, based on suppliers' recommendations.

4. On Day 5, treat cultures with maturation agent (typically TNF at 25-50ng/ml) along with GMCSF & IL-4. As needed, cultures can be given a second dose of maturation signal and harvested for analysis on the following day.
Note: Other maturation agents such as LPS and other TLR ligands or a cocktail of inflammatory agents, can also be used to induce maturation.

5. DC cultures, when observed by microscopy appear in clusters and show typical dendrite morphology.

6. In this short term protocol, mature DCs are ready for phenotype and functional analysis on Day 6 to Day 8.

Note: It is good practice to continue to feed cultures with fresh medium and cytokines until harvest and analysis.