Institut für HIV Forschung
SOP#07-00 **(Juli 2016 – BS)**

Monocyte Derived Dendritic Cell (DC2d)/Macrophages

Reagents:

Reagent	Vendor	Catalogue #	Stock Conc.
Recombinant human IL-6	ImmunoTools	11340060	1000IU/ul
Recombinant human IL-1beta (IL-1b)	R&D Systems	201-LB-005	10ng/ul
Recombinant human TNFa	ImmunoTools	11343013	10ng/ul
Recombinant human IL-4	R&D Systems	204-IL-010	800IU/ul
Prostaglandin E Synthase	R&D Systems	7627-PS-020	
GM-CSF	R&D Systems	215-GM-010	50ng/ul
M-CSF	R&D Systems	216-MC-005	100ng/ul
6-well Tissue Culture Plate, sterile	StarLab	CC7682-7506	
OctoMACS Separator	Miltenyi	130-042-108	
CD14 Microbeads, human	Miltenyi	130-050-201	
RPMI-1640	Sigma	R0883	-
Pen/Strep	Cellgro	30-001-Cl	Pen: 5000 IU/mL Strep: 5000ug/mL
L-glutamine	Cellgro	25-002-Cl	200mM; 29.2mg/mL
HEPES (1M; 238.3mg/mL)	Cellgro	25-060-Cl	1M
FBS, Heat-inactivated	Sigma	F4135	-
PBS	Sigma	D8537	-

Media:

R10

Reagent	Stock Concentration	Volume to Add	Final Concentration
RPMI 1640	-	500	-
FBS	100% (v/v)	55ml	10% (v/v)
Penicillin/Strep	Pen: 5000 IU/mL Strep: 5000ug/mL	5.5mL 5.5mL	Pen: 50 IU/mL Strep: 50ug/mL
L-glutamine	200mM	5.5mL	2mM
HEPES	1M	5.5ml	10mM

Day 1: Dendritic cell preparation (DC2d)
(slightly higher outcome, yet less mature DC's)

1. This protocol is best performed with freshly isolated PBMCs from whole blood. If working with a frozen aliquot, let cells rest 4hr in R10 prior to activation
2. Count cells
3. Isolate CD14+ cells using the Miltenyi microbead kit according using the manufacturer's protocol. Below is a simplified version
    a. Spin cells 350xg, 10min. Discard supernatant and resuspend in 80ul MACS buffer /10^7 cells
    b. Add 20ul CD14 microbeads /10^7 cells

c. Let incubate for 15 min in the refrigerator, make sure that no magnet is close
d. Wash cells with 2mL MACS buffer. Spin 350xg, 10min and discard supernatant
e. Resuspend cells in 500ul MACS buffer
f. This impacts result tremendously! Wash Ab off. Prepare MS/LS MACS column by washing column with MACS buffer once. (500ul MS, 3mL LS)
g. Apply cell suspension to column once the wash colume has traveled through
h. Collect unlabeled cells in a new FACS tube
i. Add 3x Vol MACS buffer to column to wash all unlabeled cells away
j. Remove the column from the magnet and place in new FACS tube
k. Add 500ul to column and use the plunger to force the CD14 cells into tube

4. Wash sorted CD14 cells in warm R10. Spin at 350xg, 5min and discard the supernatent
5. Resuspend cell pellet in 1-3mL of R10 and count cells.
6. Plate cells at a concentration of 1.5-3 million cells per well in a 6 well tissue culture plate in 2 mL of R10
7. Add 800 IU/mL IL-4 and 1000 IU/mL GM-CSF to the cell suspension
8. Incubate cell for 24 hours at 37° C and 5% CO_2

Day 2: Maturation of DCs

1. After 1 day of culture, add maturation cytokines directly without washing.
    a. proinflammatory cytokine maturation cocktail:
        i. 10 ng/mL TNFa
        ii. 10 ng/mL IL-1b
        iii. 1000 IU/mL IL-6
        iv. 1µM PGE2 (take 1µL of PGE2 in 2839µL Media, take from this 1µL per mL)

2. Incubate again for 24hrs at 37ºC and 5% CO_2

Generation of macrophages:

Two types of macrophages can be generated:
CD14+ cells that were isolated from Step 3 of the DC2d protocol are plated in fresh R10 media at a concentration of $2x10^6$/mL in a 6 well tissue culture plate. The media should be supplemented with either **100ng/ml M-CSF [for M-CSF derived]** or **50ng/ml GM-CSF [for GM-CSF derived]** for six days. Cells will be fed on days 3 and 5 by aspirating 80% culture volume by pipette and replacing with cytokine-containing R10.

On day 7, macrophages can be harvested for further analysis/experimentation. Vigorously pipette culture medium to dislodge weakly adherent cells and discard medium. Add fresh medium and use a sterile scraper to get macrophages into suspension.