Monocyte Cultivation

(April 2020)

MEDIA A (1 L):

1L DMEM (Invitrogen #11965-084; General Supply #108378)	
1 ml rhM-CSF (R&D #216-MC-005)	(5ug)
100 ml heat-inactivated human serum	(10%)
• Thaw in 37 ºC water bath, centrifuge 10 min. @ 3000 rpm)	
10 ml L-glutamine (Invitrogen #25030-081; General Supply #103958)	(1%)
2 ml Gentamicin (Invitrogen #15750-060; General Supply #108374)	(0.2%)
400 µl Ciprofloxacin (Sigma #17850)	(10 µg/ml)

MEDIA B (1 L):

Everything in media A but without rhM-CSF-1

PLATING MONOCYTES:

T75 flask:	30 x 10^6 cells/flask in 30 ml media A
6-well plate:	3 x 10^6 cells/well in 3 ml media A, add 2 ml media A additional on Friday.
24-well plate:	750,000 cells/well in .75 ml media A, add .75 ml media A additional on Friday.
48-well plate:	250,000 cells/well in .25 ml media A, add .75 ml media A additional on Friday.
96-well plate:	100,000 cells/well in 100 ∝l media A, add 100 ∝l media A additional on Friday.
Teflon flask (250 ml):	150 x 10^6 cells/flask in 75 ml media A. Half-exchange media on Friday.

- Plate cells immediately after receiving. Incubate at 37 ºC, 5% CO_2 $_2$
- Half-media exchange every 2 to 3 days (usually Monday, Wednesday, Friday)
- Let cells differentiate for 7 days before using for infection, stimulation or other studies
- Use media B (without mCSF) after 7 days post differentiation.