Viral infection in PBMCs

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Day -3

1. Fresh or thawed PBMCs should be resuspended in complete RPMI + 10ng/ml IL-2 at a density of $3x10^6$ cells/ml.
2. Add 3ug/ml PHA to stimulate the cells, and incubate at 37°C for three days.

Note: To make the complete RPMI buffer, add 10% heat-inactivated FBS (50ml) and 10ml of penicillin-streptomycin-L-glutamine to one 500ml bottle of RPMI. For this assay, also add 10 ng/ml IL-2. This buffer should be pre-warmed for approximately 30 minutes before use. Complete RPMI + IL-2 is used for all steps in this protocol.

Day 0

1. Tilt the flask gently to resuspend cells, remove cells/media from flask, and transfer to 50ml conical tubes. Count cells.
2. Spin at 2000rpm for 5 min.
3. Aspirate the supernatant and resuspend the cells at a density of $10x10^6$ cells/ml. For each infection, you will need 1.5mL of PBMCs at concentration of $10x10^6$ cells/mL (i.e. for two different viruses, 3ml of $10x10^6$ cells/mL will be needed).
4. To the cells, add polybrene at a final concentration of 2ug/ml. Incubate at 37°C for 30 min.
5. Spin at 2000rpm for 5 min.
6. Aspirate the supernatant, flick the tube to dislodge the pellet, and resuspend cells in fresh RPMI + 10ng/ml IL-2 media at a density of $10x10^6$ cells/ml.
7. Aliquot 1.5ml of PBMCs into 15mL conical tubes, using a separate tube for each virus or condition being tested.
8. Add virus to the cells. Viral concentrations can range from 0.1-5ng/ml (note that viral concentration is dependent on the final resuspension volume of 5ml, not the initial volume of 1.5ml).
9. Incubate for 3 hours at 37°C for 3 hours, flicking the tube to resuspend cells every hour.
10. Spin at 2000rpm for 5 min.
11. Aspirate the supernatant, flick the tube to dislodge pellet, and resuspend the PBMCs in 5mL RPMI + 10ng/mL IL-2.
12. Transfer the cells to a $25cm^2$ tissue culture flask and incubate at 37°C.

Day 3

1. Carefully remove the flasks from the incubator, making sure not to disturb the cells on the bottom of the flask. When ready to remove media, PBMCs should be a visible layer on the bottom of the flask, and not resuspended in the media.
2. Remove 2.5mL from each flask and spin down at 2000rpm for 5 min. To the flask, add 2.5ml of fresh pre-warmed RPMI + 10ng/ml IL-2 media (for a final volume of 5ml). Replace flask in 37°C incubator.
3. Aliquot the supernatant from spun-down 2.5ml samples and freeze at -80°C.

Day 7

Repeat as for day 3.

Keep infection going for as long as necessary. Time points are typically pulled at days 3, 7, 10, 14, 17, 21, 24, 28, 31, 35, etc.