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Propagation Procedure

Propagation procedure in PHA-stimulated T-cells is as follows:

R20-50 Medium (propagation medium):

RPMI 1640	500ml
FBS (heat inactivated 1hr at 56C)	110 ml (approximately)
200 mM L-Glutamine	5 ml
Penicillin/Streptomycin	5 ml (Penicillin is 5000 i.u./ml, Streptomycin is 5000 µg/ml)
HEPES buffer (1 Mstock)	6.2 ml
IL-2	280 µl of 1 x 10^5 units/ml
(Medium is approximately 50 units/ml)	

Two round-bottom 5ml tubes each containing 1 x 10^5 PBMCs stimulated with PHA 2 days before and 200 µl of viral isolate are spun for 3 hours at 37°C and 3000 rpm. The two tubes are transferred to a 25 cm^2 flask with 5 ml R20-50 medium and 5 x 10^6 PBMCs stimulated with PHA 2 days before and incubated at 37°C and 5% CO_2 in a humidified incubator for 3 to 4 days. 10 ml R20-50 medium and 5 x 10^6 PBMCs stimulated with PHA 2 to 4 days before are added and incubation is continued. At both 1 week and 1.5 weeks from infection, the culture is fed again with R20-50 by removing and replacing about half of the supernatant volume. Starting at 1.5 weeks after infection, viral growth is monitored by p24 ELISA. 2 weeks after infection and once a week subsequently, the culture is split by re-suspending the cells, removing half the volume, and replacing with an equal volume of fresh R20-50 containing 5 x 10^6 PBMCs stimulated with PHA 2 to 4 days before. At 2.5 weeks and weekly thereafter, culture is fed by removing half the volume without re-suspending cells and replacing with an equal volume of R20-50 or a greater volume if a larger volume is desired. When the p24 indicates a sufficient concentration of virus, the culture is harvested by pelleting the cells at 1500 rpm for 10 minutes and removing the supernatant with a pipet.