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 56°C): 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 M stock): 6.2 ml
- IL-2: 280 μl of 1 x 10^6 units/ml

(Medium is approximately 50 units/ml)

Two round-bottom 5ml tubes each containing 1 x 10^6 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 cm² 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% CO₂ 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.

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**ALL RECIPIENTS OF THIS MATERIAL MUST COMPLY WITH ALL APPLICABLE BIOLOGICAL, CHEMICAL, AND/OR RADIOCHEMICAL SAFETY STANDARDS INCLUDING SPECIAL PRACTICES, EQUIPMENT, FACILITIES, AND REGULATIONS. NOT FOR USE IN HUMANS.**

REV 10/30/07