Institut für HIV Forschung
SOP #19-00 **(April 2015)**

Virus Culturing Protocol from PBMC

For Elite controllers (patients with VL level below 50 copies/ml) will most likely not work due to low viral frequency .
See protocol for Elite Controllers autologus virus cultivation.

Supplies:

Fluid-resistant disposable lab coat
Exam gloves (two pairs when working in hood)
Protective eyewear if desired
Regular *sterile* P200 and P1000 tips
Sterile 25 cm^2 and 75 cm^2 flasks (T25 and T75 flasks)
Uninfected PBMCs (from donation or negative Buffy-coat)
Anti-CD3/8 antibody
R10: 500 mL RPMI-1640
5 mL Pen/Strep
5 mL L-glutamine
6.2 mL HEPES
55 mL FBS
R10-50: Add one 280-µL aliquot of 1 x 10^5 units/mL, 0.22µ filter-sterilized IL2 to one bottle R20

Item	Manufacturer	Order Number
RPMI-1640	Sigma	R0883
Pen/Strep (5000 IU Pen/ 5000ug/mL Strep)	Mediatech	30-001-Cl
L-glutamine (200mM; 29.2 mg/mL)	Mediatech	25-002-Cl
HEPES (1M; 238.3mg/mL)	Mediatech	25-060-Cl
FBS, Heat-inactivated	Sigma	F4135 (a lot tested for here)
IL2 (1 million units, dissolved per SOP#4)	Hoffmann-La Roche	Ro 23-6019
PBS	Sigma	D8537
PHA (2 mg, dissolved per SOP#5)	Remel	HA16/30852801
Anti-CD3/8 Antibody—	Dr. Johnson WONG	
Round-bottom 5-mL polypropylene FACS tubes	Falcon	352063
Round-bottom 5-mL polystyrene FACS tubes	Falcon	352058

Day 0

1. Thaw the desired vial of patient cells. See Protocol #11 Thawing.
2. After washing twice (as Thawing protocol indicate) aspirate to 200ul and add R10/50 IL-2 medium so cells are at a final concentration of 1-2 million/ml. Add anti CD3/8 to final concentration of 0.5ug/ml.
3. Same day ficoll negative blood (or Buffy) to generate activated CD4 cells for D4 – stimulate with anti CD3/8 antibody (cultivate in R10/50 medium) .

Day 4

1. Remove 1ml of the supernatant from patient CD4 cells to measure p24 levels –collect and freezer (1ml sup + 100ul triton blue)
2. Aspirate rest of the media leaving approximately 5 ml with the cells and add 20-40M negative CD4 cells from D0.
3. Add fresh R10/50 to bring the total volume up to 15 ml.

Day 6

1. Harvest 1 ml supernatant in the morning and run p24 assay. Be careful as to not take any cells, just supernatant. If the p24 level reaches 80-100ng/ml – the virus is ready to be harvested. This might happen if the VL was high on that particular sample, if not proceed with protocol.
2. If the p24 is below 80-100 aspirate the remaining medium to about 5 ml and transfer the cells to a larger T75 flask.
3. Bring the total culture volume up to 25ml with R10/50.
4. Start negative Buffy for blasts with CD3.8 for D11
5. Keep running p24 on D6,7,8 - when 80-100pg/ml harvest

Day 10

1. Mix culture and discard 10 ml of cell suspension.
2. Add 30-40M activated blasts from D6 to total volume 25ml in R10/50 media.
3. Keep running p24 every day at that point –early in the morning so you can harvest D11,12,13,14 – when 100-150 pg/ml –harvest

Day 13

1. Start negative Buffy for blasts with CD3.8 for D16

Day 16

1. Mix culture and discard 10 ml of cell suspension.
2. Add 30-40M activated blasts from D13 to total volume of 25ml in R10/50 media
3. For large volume of virus split cells to 2 T57

If it is not possible to follow that schedule precisely the whole idea is to add activated CD4 cells every 6-7 days, do not dilute culture too much although add fresh media every week (to maintain PH balance of the culture – should not be too yellow) and run p24 frequently so the virus would be harvested on the rising slope to assure the most virulent batch.

VIRUS HARVEST:

When virus reach 80-100ng/ml harvest virus free supernatant:
Spin cells in 50 ml conical (1500RPM 10minutes)
Harvest supernatant to second 50 ml conical and spin 1800 RPM 10 minutes again.
Aliquot 1ml in tubes with O-ring
Make clear labels (print them) with name of virus, date, p24 level