Institut für HIV Forschung
SOP #20-00 **(Jan 2017 - BS)**

PROPAGATION OF PRIMARY HIV-1 ISOLATES

Supplies:

Fluid-resistant disposable lab coat
Exam gloves (two pairs when working in hood)
Protective eyewear
Regular *sterile* P200 and P1000 tips
Sterile 25 cm^2 and 75 cm^2 flasks (T25 and T75 flasks)
Uninfected PBMCs (frozern or freshly prepared Buffy-coat)
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)	Sigma	0781
L-glutamine (200mM; 29.2 mg/mL)	Sigma	59202C-100ML
HEPES (1M; 238.3mg/mL)	Sigma	H0887-100ml
FBS, Heat-inactivated	Biochrom	S 0115 (a lot tested for here)
IL2 (1 million units, dissolved per SOP#04-01)	Fisher	PHC0027
PBS	Sigma	D8537
PHA (stock conc. 250ug/ml, per SOP#04-02)	Fisher	R 30852801
p24 ELISA	R&D Systems	DHP240
Cryotube inner-threaded	Oehmen	122 263

Day -3/-4

1. Prepare PHA blasts to be used for infection. Use either freshly isolated HIV- PBMCs from buffy coats or thaw a vial of HIV- PBMCs from the liquid nitrogen stock. Frozen PBMCs should be washed twice with R10 and allowed to rest for at least 4hr in the incubator
2. Resuspend HIV^- cells at $1x10^6$ cells/mL in R20 (20% FCS instead of 10%)
3. Add PHA so that final conc. of PHA is 250ng/mL. Mix thoroughly.
4. Transfer cells to either a T25 or T75 culture flask
5. Incubate at 37C, 5% CO_2 for 3-4 days

Day 0

CULTURE SET UP DAY.

- Aliquot $40x10^6$ of PHA blasts and spin them down at 500xg, 10 minutes

VIRUS ADSORPTION CYCLE #1

- While the blasts are spinning, quickly thaw a vial of virus (if your stock is 0.5 ml, two vials would be better) and add thawed virus supernatant directly to the cells by gently mixing them with a P1000 pipette. Final volume should be 1-1.5 ml
- Incubate the virus with cells in the incubator for 2 hours.
- Every 20-30 minutes gently mix cells for few seconds

- After 2 hour incubation bring the volume up to 30 ml with R10 and centrifuge for 300xg, 10min.
- Aspirate supernatant and break up the cell pellet gently by pipette mixing with the remaining media in the conical

VIRUS ADSORPTION CYCLE#2

- Quickly thaw another 1 (or 2 vials) of virus and add to the pellet of cells for 2 hours incubation (repeat VIRUS ADSORPTION CYCLE #1)

- After incubation resuspend cells in 10ml R10/50 and split into two T75 flasks. Add additional 25 ml of R10 to each flask bringing the final volume of each to 30 ml.

Day 3

Beginning on day 3, cultures are monitored for p24 production. From this point on, each culture is treated separately based on p24 data and cell density.

1. Mix the culture medium by gently shaking the flasks back and forth
2. Remove 900 ul of supernatant – run p24 ELISA on the sample (SOP #28-00)
3. If a culture has a very low p24 conc. (1-20ng/ml) then prepare PHA blasts as described above for feeding on day 6/7/8
4. If the culture has reading of >100 ng/ml culture is split (for cultures between 75-100 ng/ml, split next day):
    - Remove about 20 ml supernatant and discard. Gently mix the rest of the cell suspension and split to second flask (5-10 ml each), bring up volume to 30 ml with R10/50 medium and return the flasks to the incubator

Day 5, 6, 7

1. Monitor p24 as above.
2. Review p24 data. Harvest if p24 concentration reaches 50-100ng/ml (see "HARVEST" section below)
3. If the culture has reading of <50 ng/ml, the culture is fed (can be done the following day):
    a. Aspirate and transfer all of the cells and supe to a 50mL conical and spin at 300xg, 5min
    b. Remove and discard supernatant. Resuspend cells with fresh R10 and add $4x10^6$ PHA blasts to the flask. Return flasks to incubator

Day 10

1. Monitor p24 as above and harvest day 10 virus stocks as necessary

HARVEST

1. Cool a centrifuge to 4C
2. Prepare the tubes that the virus will be collected in. We store 1mL aliquots of viral supernatent and use 1.5mL cryotubes with inside threading (same that are used for freezing down cells).
3. Spray the bags with the eppy tubes and lids with 70% EtOH and place inside the hood. Place a lid on each tube to be used.
4. Create labels for the tubes. The labels should have the HIV strain/batch, p24 concentration, and date. Prepare an extra label for the box they will be stored in.
5. Label each tube prior to harvesting virus. The virus from this point on should be kept cold as often as possible. Use a bucket full of ice for storing the virus conicals between spins.
6. Get a box from the freezer room to store the aliquots in. Place the label(s) of the virus(es) that are going into the box on the front of the lid. Place the box in the -20C until needed.
7. Take the virus cultures from the incubator to the hood. Transfer everything from the culture flask to a 50mL conical.

8. Clarify supernatent by centrifugation. Initially, spin at 400xg, 10 minutes and 4C. Transfer the entire supernatant to a fresh 50 ml conical and spin again at 1100xg, 10 minutes, 4C.
9. During the spin, add ~20mL warm R10 media to the cells and resuspend them. Place back in the incubator to be frozen down later
10. After the spin is complete, aliquot cell free virus (1ml/tube) into prepared tubes
11. Keep aliquots cold and transfer them to the box(es) you placed in the -20C in step 6. Put boxes in the "biological hazard" transfer container and transport them to the freezer room and place them in the -80C, third shelf, first rack.
12. Update the "HIV Virus_Plasmid Log" file on the server with the number of aliquots, final concentration, and location
13. Update the -80C map so we know where they were stored
14. Freeze infected PBMCs according to SOP #08-01 on the server. Freeze $20x10^6$ cells per vial and on the label record the HIV strain/batch, number of infected cells, and the date. Once frozen, transfer cells to Rack T in the liquid nitrogen tank and update the log on the server.