Amplification of HIV-1 Infectious Virus in BL3 Lab

Xin Wang*

Department of Genetics, Stanford University, Stanford, USA
*For correspondence: xinwang73@hotmail.com

[Abstract] This method is used for making high titer human immunodeficiency virus type-1 (HIV-1) virus stock for subsequent infection assays. The amplification of T-tropic HIV-1 virus (IIIB strain) uses the $CD4^+$ T cell line H9.

Materials and Reagents

1. $CD4^+$ T cell line H9 cells (ATCC HTB-176)
2. Fetal bovine serum (FBS) (Thermo Fisher Scientific, catalog number: SH30071.03 HI)
   Note: This particular FBS has been tested by the author, but may be substituted with FBS from different suppliers as desired by users.
3. Penicillin-Streptomycin liquid (Life Technologies, Gibco®, catalog number: 15070-063)
4. Cell culture media: RPMI1640 (Life Technologies, Gibco®, catalog number: 11875-093) (see Recipes)

Equipment

1. Bench-top centrifuges
2. CO_2 incubator
3. -80 °C freezer
4. 50 ml conical tubes (BD Biosciences, Falcon®, catalog number: 35-2070)
5. 5, 10, 25-ml pipet (BD Biosciences, Falcon®, catalog number: 35-7501, 35-7554, 35-7556)
6. T-75, T-175 cell culture flask (BD Biosciences, Falcon®, catalog number: 35-3136, 35-3112)
7. CryoTube Vials (NUNC, 377267)

Procedure

Day 1

1. Dilute 2 x 10^6 uninfected H9 cells in 2 ml of culture medium (final concentration 1 x 10^6 cells/ml) in a 50-ml falcon tube.
2. Add 1 ml of HIV-1 virus (IIIB strain) to the falcon tube.
3. Shake gently every 15 min for 1 h.
4. Pipet this miniculture into 50 ml of culture medium in a T-75 cell culture flask.
5. Incubate at 37 °C for 4 days.

Day 5

6. Add 175 ml fresh media to each of 2 new large falcon T-175 cell culture flasks.
7. Add 25 ml of 4-day culture to each flask.
8. Incubate at 37 °C for 3 days.

Day 8

9. HIV-1 infected cells (400 ml) were transferred into 10 x 50-ml falcon tubes using a 25-ml pipet (40 ml per tube).
10. Centrifuge in secondary containers at 1,300 rpm for 8 min.
11. Carefully remove supernatant using the vacuum line with pipet tip attached to a 10-ml pipet (fill collection flask with bleach).
12. Add 2 ml of fresh media to each tube and loosen the cell pellets.
13. Collect all cells in 2 tubes and rinse the other 8 tubes with fresh media and add into those 2 tubes.
14. Add fresh media to each of these 2 tubes to a final volume of 40 ml.
15. Centrifuge in secondary containers at 1,300 rpm for 8 min.
16. Remove supernatant.
17. Add 10 ml of fresh media to each flask and loosen cell pellet.
18. Combine into one tube.
19. Beat up cells for 20 sec using tube rack.
20. Centrifuge in secondary containers at 2,000 rpm for 8 min.
21. Transfer supernatant using pipet into a fresh 50-ml falcon tube (Supernatant 1).
22. Add 10 ml of fresh media to tube with cell pellet and loosen cell pellet.
23. Beat up cells again using tube rack.
24. Centrifuge in secondary containers at 2,000 rpm for 8 min.
25. Transfer supernatant using pipet into a fresh 50-ml falcon tube (Supernatant 2).
26. Tranfer Supernatant 1 and 2 into small CryoTube vials using a 5-ml pipet (1 ml each tube) (label tubes with IIIB, date and either Sup1 or Sup 2).
27. Store in -80 °C freezer.

Notes

The experiment using HIV-1 needs to be done in a BSL-3 lab and the personnel require special training for working with HIV-1 virus.

Recipes

1. Culture medium (500 ml)

RPMI1640 medium	500 ml
FBS	50 ml
Penicillin-streptomycin	5 ml

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References

1. Dutschman, G. E., Grill, S. P., Gullen, E. A., Haraguchi, K., Takeda, S., Tanaka, H., Baba, M. and Cheng, Y. C. (2004). Novel 4'-substituted stavudine analog with improved anti-human immunodeficiency virus activity and decreased cytotoxicity. *Antimicrob Agents Chemother* 48(5): 1640-1646.

