

Protocol for testing replication competent lentivirus (RCL)

A major concern regarding the biosafety of lentivirus-based vectors is the replication competent lentivirus/genome (RCL). The production of VSV-G pseudotyped lentivirus in the RNAi Core was carried out by using the two and half generation vector system, a three plasmids co-transfection system (self-inactivated lentivirus transfer vector, pMD.G (G protein of VSV), and pCMV Δ R8.91 (support HIV gag-polymerase, Rev and tat protein)). To avoid the potential of producing RCL, two approaches were performed in the Core: (i) a large amount of package plasmids (\sim 50-100 mg stock of pMD.G and pCMV Δ R8.91) was prepared as a stock in a non-HIV-1 working environment to ensure that those vectors would not contaminate with HIV-1 infectious constructs or related HIV-1 constructs); (ii) we checked the RCL every 6-12 month by monitoring p24 production. In this assay, we employed a suspension cell, SUP-T1, to detect the release of p24 in culture fluid. SUP-T1 was reported to be fully infected by lentivirus. In addition, suspension culture system allows us to infect cells with large amounts of lentivirus in one assay (one flask). This procedure protects the worker who performs the assay. The following protocol is currently used in the Core for RCL assay.

Day 1

1. 1×10^7 SUP-T1 cells were pelleted and resuspended with 10ml RPMI1640 complete medium (CM) containing 16 μ g/ml polybrene in a 50-ml centrifuge tube.
2. $2-4 \times 10^8$ shLuc#221 lentiviruses (titer: $2-4 \times 10^7$ /ml, volume: 10ml) were added.
3. After mixing, mixtures were aliquoted into a 6-well plate. Centrifuged at 1100g for 30min at 37°C (spin infection).
4. The cell mixtures were transferred to a T75 flask and cultured at 5% CO₂ incubator, 37°C.

Day 2

5. The cell suspension was collected into a 50-ml tube, centrifuged at 300g for 3 min, supernatant was discarded, and cell pellet was resuspended with 15ml RPMI1640 CM containing 2 μ g/ml puromycin in a T75 flask, incubate at 37°C, 5%CO₂.

Day 4

6. Three days after infection, half of the cell suspension was pelleted, and 1 ml of supernatant was collected and filtered through a 0.45 μ m filter and stored at -20°C for RCL assay (label as RCL1).

7. Remaining supernatant was aspirated and the cell pellet was resuspended in T75 flask with 10ml fresh RPMI1640 CM containing 2ug/ml puromycin, cultured at 37°C, 5%CO₂ incubator.

Day 7-19

8. Repeat steps 6-7 every 2 to 3 days for a period of 7 passages (~ 2 weeks, supernatants were labeled as RCL2 to RCL7).

Culture condition:

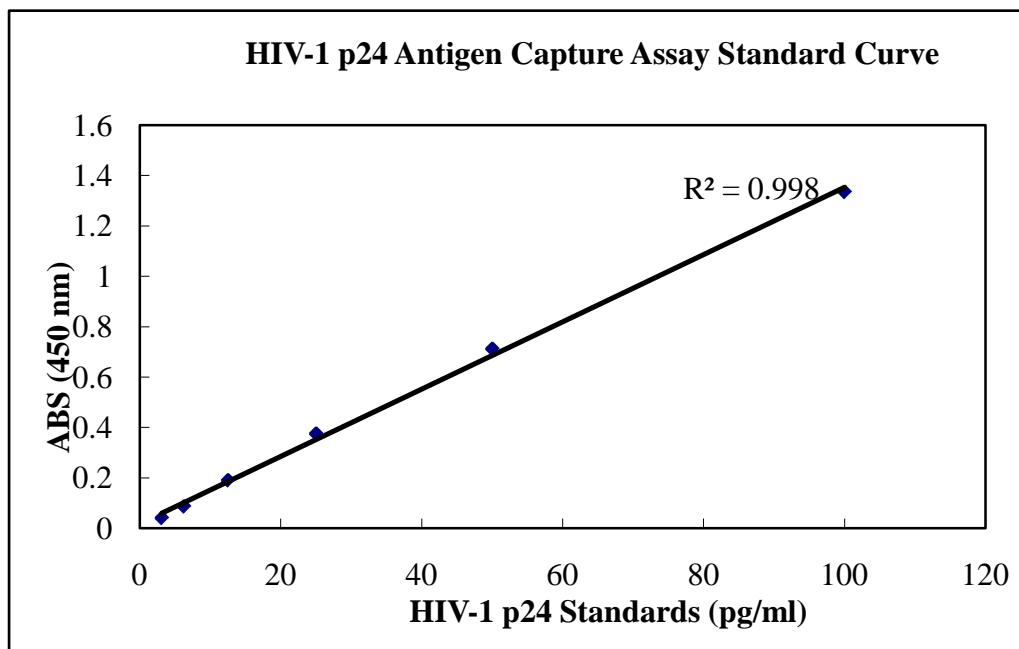
SUP-T1 cells were cultured in T75 at a cell density of 1×10^6 /ml in RPMI1640 + 10%FBS + 1XP/S (RPMI1640 CM).

RCL assay (by measuring the amount of releasing p24 using ELISA):

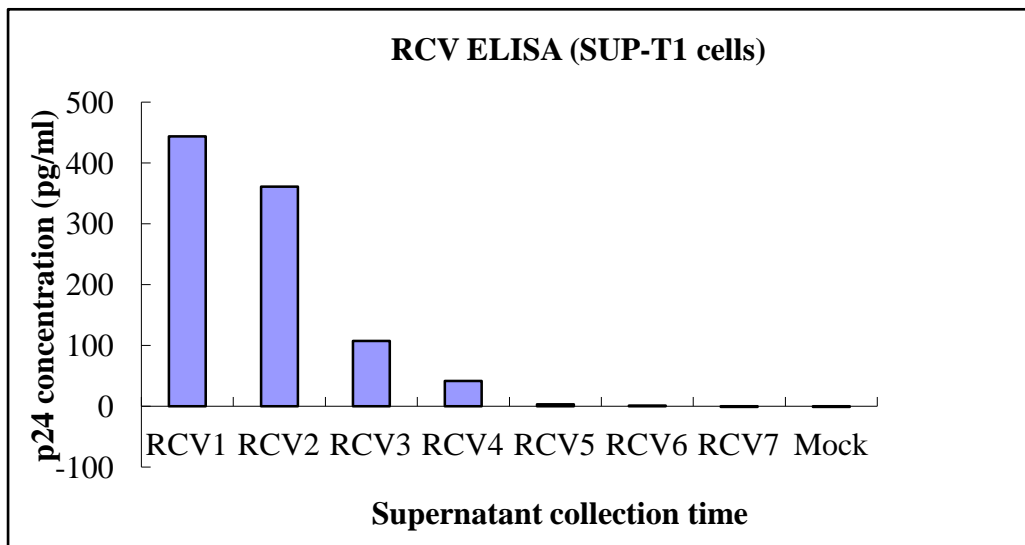
Measurement of p24 concentration was performed using the HIV-1 p24 Antigen Capture Assay (Catalog#5421 Advanced BioScience Laboratories, Inc.) in accordance with manufacture's instruction.

Example of testing result:

1. Standard curve



2. Results



The p24 values of RCL1 to RCL4 were derived from 20 and 40 folds diluted supernatant. Other samples were assayed by using undiluted supernatant. All samples are in duplicate.