Standard Operating Procedure
for
TITERING YOUR LENTIVIRAL STOCK

1.0 PURPOSE

This procedure describes how to determine the titer of lentiviral stocks.

2.0 SCOPE (Should include which Cores this SOP applies to)

This procedure applies to all personnel who wish to titer the lentiviral stock.

3.0 PROCEDURE

1. Dilute lentiviral stock with assay dilute solution at a ratio of 1:1000. Add 50 µl of Lysing Buffer into 450 µl specimen and mix well.
2. Wash each well of the selected number of strips 6 times with 300 µl of 1× Plate Wash Buffer. Aspirate wash buffer and strike the strips on a pad of absorbent towels until no droplets remain in the wells. Note: Do not allow washed plates to dry completely prior to sample addition.
3. Pipet 200 µl of standards #1-6 and specimens from step 1into duplicate wells. Leave one well empty as a substrate blank.
4. Cover microplate with a plate sealer and incubate two hours or overnight at 37°C.
5. Aspirate and wash plate as described in Step 2.
6. Pipet 100 µl of reconstituted HIV-1 p24 Detector Antibody into each well, except the substrate blank. Cover the microplate with a sealer and incubate for 1 hour at 37°C.
7. Aspirate and wash plate as described in Step 2.
8. Dilute Streptavidin peroxidase with assay dilute at a ratio of 1:100. Pipet 100 µl of the Streptavidin-Peroxidase Working Solution into each well except the substrate blank. Cover the microplate with incubate for 30 minutes at 37°C.
9. Aspirate and wash plate as described in Step 2.
10. Dilute substrate with substrate buffer at a ratio of 1:100. Pipet 100 µl of Substrate Working Solution into all wells and incubate uncovered for 30 minutes at room temperature (18°- 25°C). A blue color will develop in wells containing viral antigen.
11. Stop the reaction by pipetting 100 µl of Stop Solution into each well. The color will change from blue to yellow.
12. Within 15 minutes, read the optical density of each well at 450 nm using a microplate reader.
13. Use excel to get a linear equation of p24 Antigen Standard. Determine the concentration of p24 antigen in specimens and then calculate the concentration of lentivirus particle (1 ng P24= 1×10^5 physical particle (PP)).

Note: For the test to be valid, the result must meet the following criteria:
- The mean optical density of the 0 pg/ml standard and the substrate blank must be less than 0.200.
- The mean optical density of the 62.5 pg/ml standard must be greater than or equal to 0.500.

Table 1
Preparation of HIV-1 p24 Antigen Standard

Standard number	Concentration of HIV-1 p24 (pg/ml)	HIV-1 p24 Antigen Standard (µl)	Assay Diluent (µl)
1	125.0	50	950
2	62.5	500 of #1	500
3	31.3	500 of #2	500
4	15.6	500 of #3	500
5	7.8	500 of #4	500
6	0.0	500 of #5	500

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