I. Introduction

This protocol-at-a-glance is not intended for first-time users. If you have not used this kit before, please refer to the Lenti-X p24 Rapid Titer Kit User Manual.

NOTE: Lysis Buffer and Wash Buffer (20X) no longer include a blue dye that was added to these reagents in previous lots of the Lenti-X p24 Rapid Titer Kit. This update was confirmed to have no impact on performance of the kit.

II. Protocol

A. Wash Buffer Preparation

Prepare 1X wash buffer by diluting 1 part Wash Buffer (20X) with 19 parts distilled or deionized water. If the kit will be utilized over a period greater than 4 weeks, then prepare only enough working strength wash buffer for immediate needs. Each strip of 8-wells can be adequately washed with ~60 ml of working strength wash buffer.

B. Preparing Dilutions for the p24 Standard Curve

1. Prepare a working strength p24 positive control stock solution by diluting 20 µl of the p24 Control (10 ng/ml) into 980 µl of fresh complete tissue culture medium (e.g. DMEM containing 10% FBS), for a 1:50 dilution. This will produce a 200 pg/ml stock solution.
2. Using the 200 pg/ml stock and complete tissue culture medium as the diluent, make a series of four additional standard dilutions of 100, 50, 25, and 12.5 pg/ml. Dispense 500 µl of media into each of four labeled tubes. Add 500 µl of the 200 pg/ ml stock into the 100 pg/ml tube, mix, and using a fresh pipet tip, transfer 500 µl of this 100 pg/ml solution into the 50 pg/ml tube and mix. Repeat similar transfers for the 25 and 12.5 pg/ml tubes.

C. Assaying Your Lentiviral Supernatants

1. Allow all reagents to reach room temperature (18–25°C).
2. Select a sufficient number of 8-well strips to accommodate all standards, test specimens, controls, and culture medium blanks in duplicate. Fit the strips into the holding frame. Label wells according to specimen identity using the letter/number cross reference system molded into the plastic frame.
3. Dispense 20 µl of lysis buffer into each well.
4. Dispense 200 µl of each standard curve dilution, supernatant sample, and blank into appropriately labeled duplicate wells.
5. Incubate at 37(±1)°C for 60 (±5) minutes.
6. Aspirate the contents of the wells, and wash the microtiter plate as described in the user manual (Section VI).

7. Dispense 100 µl of Anti-p24 (Biotin conjugate) detector antibody into each well.

8. Incubate at 37(±1)°C for 60 (±5) minutes.

9. Aspirate the contents of the wells, and wash the microtiter plate as described in the user manual (Section VI).

10. Dispense 100 µl of Streptavidin-HRP conjugate into each well.

11. Incubate at room temperature (18–25°C) for 30 (±5) minutes.

12. Aspirate the contents of the wells, and wash the microtiter plate as described in the user manual (Section VI).

13. Without delay, dispense 100 µl of Substrate Solution into each well. A multichannel pipet should be used for best results.

14. Protect the plate from direct light/sunlight, and incubate at room temperature (18–25°C) for 30 (±2) minutes.

15. Stop the reaction by adding 100 µl of Stop Solution to each well including the culture medium blank. The blue solution should change to a uniform yellow color. Ensure that the undersides of the wells are dry and that there are no air bubbles in the well contents.

16. Immediately after adding the Stop Solution, read the absorbance values at 450 nm using a microtiter plate reader blanked on the negative control well.

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