I. Introduction

The **Lenti-X™ p24 Rapid Titer Kit (Single Wash)** (Cat. No. 631476) provides a quick assay for determining the titer of any HIV-1-based lentiviral supernatant. The assay utilizes an Enzyme Linked Immunosorbent Assay (ELISA) to detect the presence of p24 in a lentiviral sample.

The following information is provided as a high-level overview of the protocol to perform the workflow. For more detailed information, please see the Lenti-X p24 Rapid Titer Kit (Single Wash) User Manual.

NOTE: For your safety, guidelines related to working with lentivirus is captured in this document in Section IV. Additional biosafety information can be found in the User Manual, "Biosafety Level 2".

II. Required Materials

This protocol applies to the following Takara Bio products:

- Lenti-X p24 Rapid Titer Kit (Single Wash) (Cat. No. 631476)

Additional materials required:

- Latex gloves, safety glasses, and other appropriate protective garments
- Biohazard infectious waste containers
- Micropipettes for delivering volumes of 2 µl, 20 µl, 100 µl, 200 µl, and 1,000 µl
- Filtered pipetting devices for 1 ml or larger pipettes
- Automatic microtiter plate washer or a vacuum line fitted with a vacuum pump trap to collect liquid
- Absorbent paper towels
- Microtiter plate reader with 450 nm filter
- Standard laboratory equipment

III. Protocol Overview

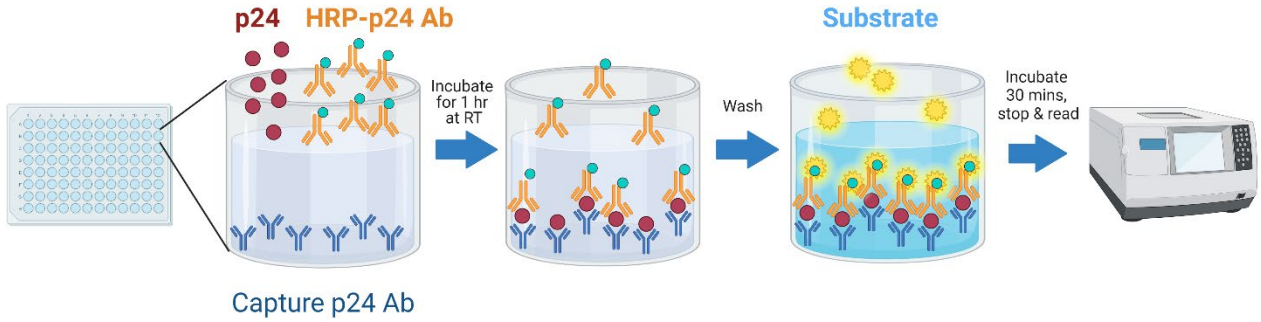

Figure 1. The Lenti-X p24 Rapid Titer Kit (Single Wash) workflow.

IV. Safety Guidelines

For your safety and the safety of others around you, it is imperative to fully understand the potential hazards of working with recombinant lentiviruses and the necessary precautions for their use in the laboratory.

For more information on Biosafety Level 2 agents and practices, download the following reference:

CDC & NIH. Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition | CDC Laboratory Portal | CDC. *U.S. Dep. Heal. Hum. Serv.* (2020). at https://www.cdc.gov/labs/BMBL.html

V. Protocol

IMPORTANT:

- Please read the entire protocol before starting.
- Reference the User Manual for general requirements and sample recommendations for the protocol.

1. Prepare 1X wash buffer by diluting 1 part Wash Buffer (20X) with 19 parts distilled or deionized water. We recommend that fresh wash buffer be prepared before each assay. Prepare only enough working strength wash buffer for what is immediately needed.
2. Allow all reagents to reach room temperature (18–25°C).
3. Prepare p24 stock solution by adding 1.25 ml of Reconstitution Buffer to p24 Control (500 ng, lyophilized powder). Vortex for 5 sec.
4. Prepare the p24 standard (1,600 pg/ml concentration) by dispensing 996 µl of Diluent Buffer into a 2 ml centrifuge tube then adding 4 µl the p24 control stock solution. Vortex for 5 sec.

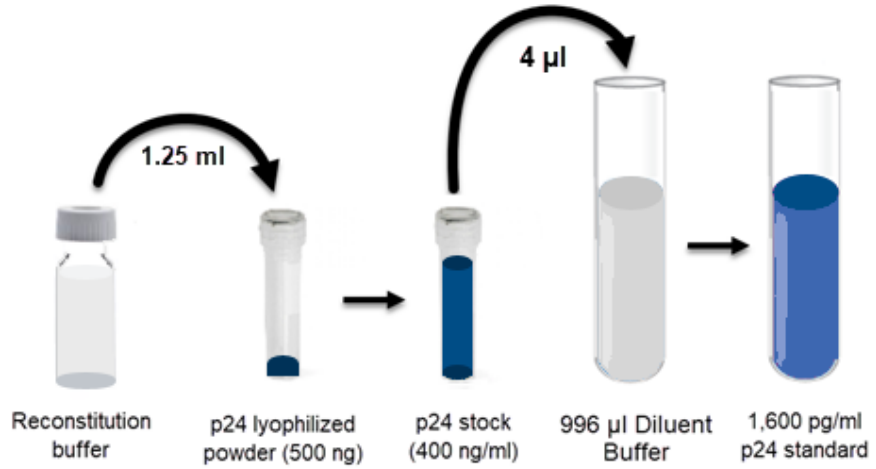

Figure 2. Preparing the p24 stock solution and 1,600 pg/ml p24 standard solution.

5. Label and dispense 500 µl of Diluent Buffer into five 2 ml centrifuge tubes for the 800 pg/ml, 400 pg/ml, 200 pg/ml, and 100 pg/ml standards, and the negative control (0 pg/ml). Set aside the negative control tube.
6. Prepare the p24 dilution series by pipetting 500 µl of the 1,600 pg/ml solution into the 800 pg/ml tube. Vortex for 5 sec.
7. Repeat Step 6 three times, pipetting 500 µl from the 800, 400, and 200 pg/ml dilutions respectively for each subsequent dilution (Figure 3, next page).

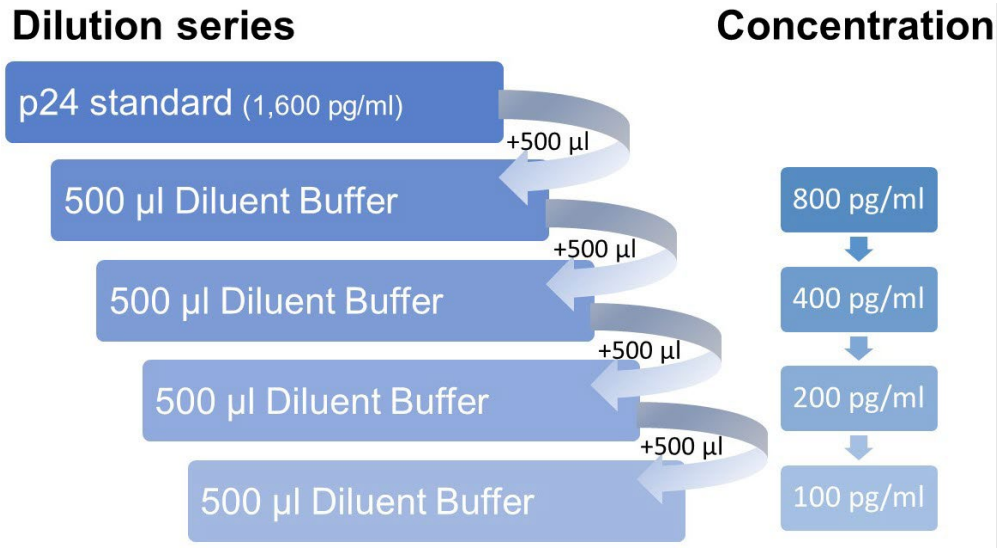

Figure 3. Preparing the p24 curve serial dilutions.

8. Prepare sample dilutions. We recommend making several serial, tenfold dilutions to generate at least one dilution in the range of the standard curve. Mix dilutions thoroughly before assaying or diluting them further.
9. Select enough 8-well microtiter strips to accommodate all standards, test specimens, controls, and Diluent Buffer (NTCs) in duplicate.
10. Fit the microtiter strips into a holding frame.
11. Assign wells based on specimen identity.
12. Prepare new 2 ml centrifuge tubes labeled for all intended replicates of the p24 standards and sample dilutions.
13. Add 100 µl of the p24 standards or sample dilutions to its respective labeled 2 ml centrifuge tube.
14. Add 20 µl of Lysis Buffer to each tube then mix.
15. Add 100 µl of the Anti-p24 (HRP conjugate) to each tube then mix.
16. Dispense 200 µl of each test sample into the designated wells of the ELISA plate assigned for the p24 standard and sample.
17. Incubate the used microtiter strips at room temperature (18–25°C) for 60 min ± 5 min.
18. Aspirate the contents of the wells and wash the microtiter plate either via automatic or manual plate washing, following the appropriate protocol detailed in the user manual. Wash/rinse a total of six (6) times.
19. After washing, without delay, use a multichannel pipette to dispense 100 µl of TMB Substrate Solution into each well.
20. Protect the plate from direct light/sunlight and incubate at room temperature (18–25°C) for 30 ± 2 min.
21. Stop the reaction by adding 100 µl of Stop Solution to each well.
22. Mix on a plate shaker for 5–10 sec or tap lightly. The blue solution should change to a homogeneous yellow color in each well.
23. Ensure that the undersides of the wells are dry and that there are no air bubbles in the well contents.
24. Immediately read the absorbance values at 450 nm using a microtiter plate reader.
25. Generate your p24 standard curve from the p24 standard dilutions.
26. Compare your sample results to the standard curve. Multiply each result by its dilution factor to determine the correct p24 value in the original sample.

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