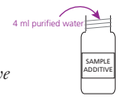
QUICK GUIDE TO THE EXAVIR LOAD VERSION 3 PROCEDURE

Quantitative Determination of HIV Reverse Transcriptase Activity

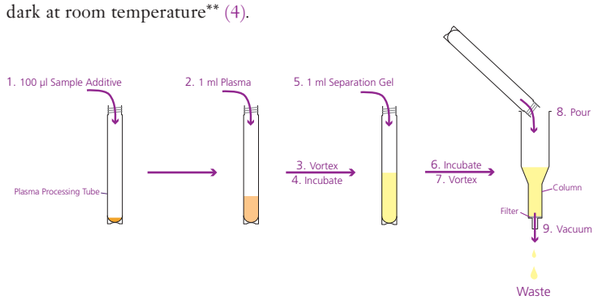
DAY ONE

1. Collect and thaw components, controls and samples.

2. Add 4 ml water to *Sample Additive*. Vortex.



Label 32 Plasma Processing Tubes. Add 100 µl *Sample Additive* to each tube (1). Add 1 ml plasma sample or control to each tube (2). Add caps, vortex (3) and incubate for 20 min. in the dark at room temperature** (4).



3. Prepare the Separation equipment (see fig. A). Label 32 Columns and Storage Tubes.

4. After 20-min. incubation - add 1 ml *Separation Gel* to each tube (5). **Shake *Separation Gel* bottle between each transfer.** Mix for 30 min. at room temperature** (6).

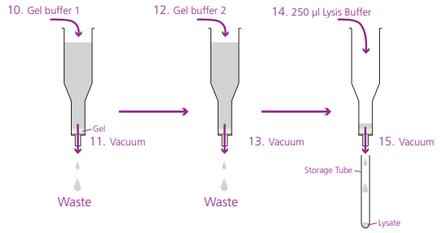
5. Prepare buffers during the incubation:

Add *Gel Buffer 1 conc* to 2-litre bottle. Rinse *Gel Buffer 1 conc* bottle with 100 ml water and add to 2-litre bottle. Add 1100 ml water to the 2-litre bottle. Mix.

Add *Gel Buffer 2 conc* and 260 ml water to 250-ml bottle. Mix.

Dissolve the *Lysis Buffer Additive* in 5 ml *Lysis Buffer*. Vortex and transfer back to *Lysis Buffer* bottle. Vortex.

6. After 30-min. incubation, vortex each tube (7) and pour into corresponding Column (8). Apply vacuum and suck gels dry (9). Wash gels four times with Gel buffer 1 (10). Apply vacuum, and suck gels dry (11).



7. Wash gels 1 time with all Gel buffer 2 (12). Suck gels completely dry before proceeding (13).

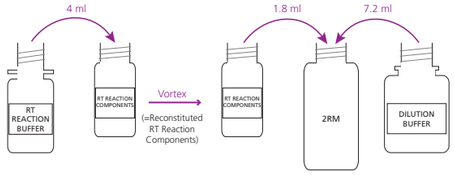
8. Connect the Lysate Collector to the Waste Container and put the Collector Tube Rack with the Storage Tubes into it. **Make sure that the valve is open and that the Vacuum Pump is turned off.** Move the Column Holder to the Lysate Collector.

Vortex *Lysis Buffer*. Add 250 µl *Lysis Buffer* to each Column (14). Wait 2 minutes. Suck the gels dry by turning on the Vacuum Pump and closing the valve for 7 seconds (15). Open the valve and close it again for 7 seconds.

Move the Storage Tubes to the Storage Tube Rack.

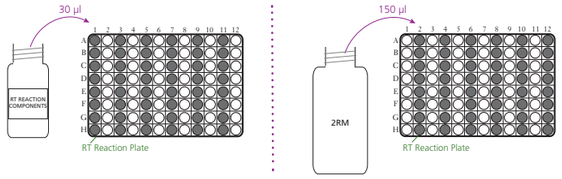
9. Dissolve the *Dilution Buffer Additive* in 5 ml *Dilution Buffer*. Vortex and transfer back to *Dilution Buffer* bottle. Vortex.

10. Add 4 ml *RT Reaction Buffer* to *RT Reaction Components*. Vortex. Mix 1.8 ml dissolved *RT Reaction Components* and 7.2 ml *Dilution Buffer* in an empty tube/bottle (2RM).



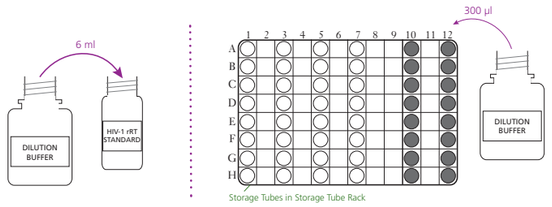
Transfer 30 µl dissolved RT Reaction Components to rows 1, 3, 5, 7, 9 and 11 of the *RT Reaction Plate*.

Transfer 150 µl 2RM to rows 2, 4, 6, 8, 10 and 12 of the *RT Reaction Plate*.

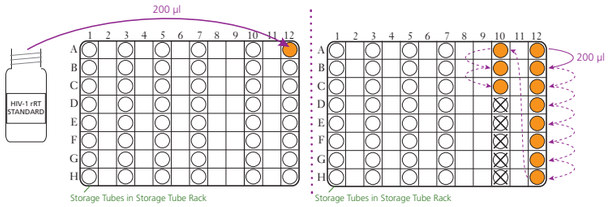


11. Add 6 ml *Dilution Buffer* to *HIV-1 rRT Standard*. Vortex.

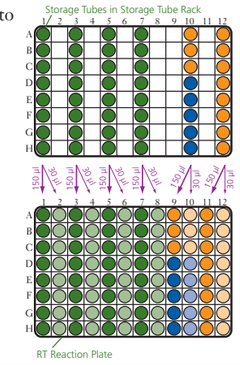
Add 300 µl *Dilution Buffer* to 16 empty Storage Tubes, positioned in rows 10 and 12 of the Storage Tube Rack.



Make a serial dilution in Storage Tubes A12 to C10 by transferring 200 µl dissolved *HIV-1 rRT Standard* to Storage Tube A12. Change tip and mix 5 times and transfer 200 µl to Storage Tube B12 etc, all the way to H12 and then continuing to A10, B10 and C10.



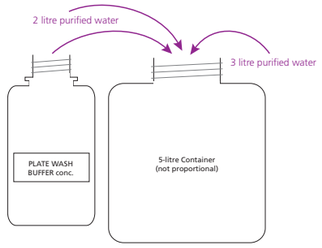
12. Add lysates, buffer blanks and standard to *RT Reaction Plate* in 150- and 30-µl portions. Seal *RT Reaction Plate* and incubate for 18 to 24 hours at 33°C.



DAY TWO

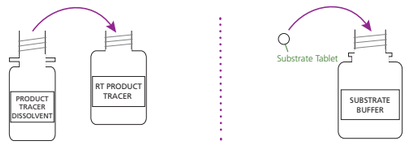
13. Collect and thaw components.

14. Mix 2 litres of water and *Plate Wash Buffer conc* in the 5-litre container. Adjust to 5 litres with water. Use some of the water to rinse out the buffer bottle and add to the container. Mix thoroughly. Pour 0.5 litre into 4 Wash Buckets.



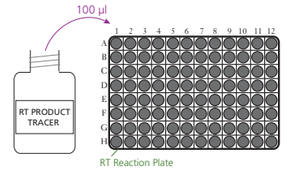
15. Dissolve *RT Product Tracer* in *Product Tracer Dissolvent*. Vortex.

16. Add *Substrate Tablet* to *Substrate Buffer*. Keep in dark until use.



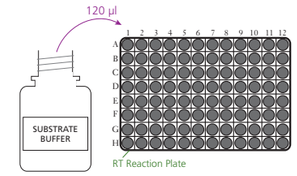
17. Collect *RT Reaction Plate* from incubator. Remove adhesive tape and secure strips with rubber band. Empty plate. Wash 15 times in bucket 1 and 2. Wash 15 times and soak for 5 min. in bucket 3 and 4. Leave plate to dry upside-down for 5 min. Rinse buckets.

18. Add 100 µl *RT Product Tracer* to all wells in *RT Reaction Plate*. Seal *RT Reaction Plate* and incubate for 90 min. at 33°C.



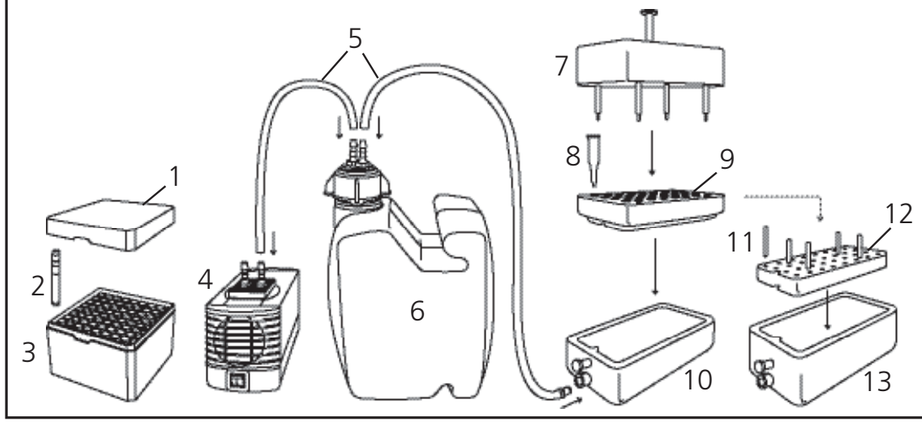
19. Pour 0.5 litre Plate wash buffer into 4 Wash Buckets. Collect *RT Reaction Plate* from incubator. Remove adhesive tape and secure strips with rubber band. Empty plate. Wash 15 times in bucket 1 and 2. Wash 15 times and soak for 5 min. in bucket 3 and 4. Remove foam from bucket 4. Take out plate and leave to dry upside-down for 5 min.

20. Vortex Substrate buffer. Add 120 µl Substrate buffer to each well in *RT Reaction Plate*. **Make sure no bubbles are present in wells.** Incubate in dark for 10 minutes at room temperature**. Read *RT Reaction Plate* at A405. Incubate in dark for 2 to 3 hours. Read. Incubate in dark for 16 to 24 hours. Read.



21. Process data in the ExaVir Load Analyzer.

FIGURE A: EXAVIR LOAD SEPARATION EQUIPMENT AND CONSUMABLES



1. Sample Box Lid
2. Plasma Processing Tube
3. Sample Box
4. Vacuum Pump
5. Vacuum Tubing
6. Waste Container
7. Buffer Dispenser
8. Column
9. Column Holder
10. Waste Collector
11. Storage Tube
12. Collector Tube Rack
13. Lysate Collector

Referring to IfU 21130-05

For a detailed description of each step, please refer to the ExaVir Load instructions for use.

**Room temperature = 18 to 33°C = 64 to 91°F