Protein Science - PEL-00012 Operation of ViroCyt

Purpose

To detail how to titer baculovirus using the ViroCyt 2100.

Materials and Equipment

- ViroCyt 2100
- 70% isopropyl alcohol (IPA)
- Startup/shutdown (SSR) vial
- Cleaning verification (CVF) vial
- Inter-sample wash (ISW) vial
- Performance validation stain (PVS) vial
- Sample dilution buffer
- Combo dye
- Combo dye dilution buffer
- Acetonitrile
- Baculovirus stock to titer
- ViroCyt sheath fluid
- ViroCyt wash fluid
- ViroCyt sample vials
- 1.7 mL microcentrifuge tubes
- Biological safety cabinet
- P1000 pipette
- P200 pipette
- P20 pipette

Procedures

A. Preparation

1. Ensure the biological safety cabinet is ready for operation according to the procedure in PEL-00026.
2. Spray the items below with 70% IPA and place them in the biological safety cabinet:
   a. Virus(es) to be titrated
   b. Combo dye
   c. Combo dye buffer
   d. Acetonitrile
   e. ViroCyt sample vials
   f. Sample dilution buffer
3. Label two 1.7 mL tubes per virus to be titrated (one with the COG ID and “1:10,” and one with the COG ID and “1:500”).
4. Add 90 µL of sample dilution buffer to each tube labeled 1:10.
5. Add 245 µL of sample dilution buffer to each tube labeled 1:500.
6. Aseptically add 10 µL of each virus to be titrated into its corresponding 1:10 vial.
7. Cap tubes and vortex them for 10 seconds.
8. Remove 5 µL from the 1:10 dilution and add it to the appropriate tube labeled 1:500.
9. Cap and vortex the 1:500 tube briefly.
10. Label 2 ViroCyt sample vials with the COG ID for each virus to be titrated.
11. Add 120 µL of the 1:500 diluted virus to the appropriately labeled ViroCyt sample tube.
12. Add 5 µL of acetonitrile to the combo dye tube (1 combo dye vial is enough for 4 viruses).
13. Incubate combo dye and acetonitrile for 5 minutes.
14. After 5 minutes, add 500 µL of combo dye buffer to the combo dye vial, cap it, and mix by inverting it 10 times.
15. Add 60 µL of prepared combo dye to each ViroCyt sample vial and cap the vials.
16. Vortex sample vials for 5–10 seconds and store them in a dark place at room temperature for a minimum of 30 minutes.

B. ViroCyt Startup

1. Ensure the ViroCyt sheath fluid and wash fluid containers are full. If they aren’t, fill them with the appropriate solution.
2. Ensure the waste has been emptied. If it isn’t, empty the waste container into Wescodyne waste in the biological safety cabinet.

3. Ensure that an SSR vial is in the sample port. If it isn't, place a fresh SSR vial in place.
4. Attach tubing from the air regulator to the air inlet at the back of the ViroCyt 2100.
5. Turn on the air supply from the bench and ensure that air flow is between 15–20 psi as measured by the attached regulator.
6. Turn on the ViroCyt 2100 using the switch on the front of the machine.
7. Turn on the attached laptop and start the control software by double-clicking the ViroCyt 2100 icon on the desktop.
8. Check the boxes corresponding to air flow, fluid levels, and SSR vial.
9. Click "Start ViroCyt."
10. The ViroCyt program will launch and proceed with a system wash and warm-up, taking 30 minutes.
11. Once the ViroCyt system wash and warm-up is complete, remove the SSR vial and replace it with the ISW vial.
12. Click on "ISW" and allow the system to wash.
13. Once the wash has completed, remove the ISW vial and replace it with the CVF vial.
14. Click the CVF button to make sure the system is clean and ready.
15. If the CVF returns "PASS," move to the next step. If it doesn't, proceed to Troubleshooting, A. CVP Step Failure.
16. Replace the CVF vial with the PVS vial and click "PVS."
17. If the PVS returns "PASS system," move to the next step. If it doesn't, proceed to Troubleshooting, B. PVS Step Failure.
18. Remove the PVS vial, replace it with the ISW vial, click "ISW," and allow the system to wash out the PVS.
19. Remove the ISW vial, replace it with the CVF vial, and click "CVF."
20. If CVF passes, the machine is now ready to process samples (see Procedures, C. Titer Measurements). If it fails, move on to Troubleshooting, A. CVP Step Failure.

C. Titer Measurements

1. Insert the prepared sample vial and click "New Sample."
2. Enter the COG plasmid ID under "Name," select "Combo Dye" under "Method," and enter "500" under "Dilution Factor." Finally, click "Measure."
3. Each sample vial can provide two readings, so repeat step C.2 until four readings for each plasmid ID have been generated.
4. After all measurements for that vial have been recorded, remove the vial, insert the ISW tube, and click "ISW."
5. Once the wash is complete, remove the ISW vial, insert the CVF vial, and click "CVF."
6. If the CVF passes, move on to the next sample and repeat steps C.1–C.5 for each sample.
7. Once the CVF on the last sample has passed and all samples have been tested, proceed to Procedures, D. Shutdown.

If a CVF fails at any point in Procedures, C. Titer Measurements, proceed to Troubleshooting, A. CVF Step Failure.

D. Shutdown

1. After all samples have been measured and the ISW and CVF have been performed and have passed, insert the SSR vial and select "Shutdown."
2. The system will perform a shutdown wash and cooldown that takes ~20 minutes.
3. After the shutdown wash and cooldown, the system will display that it is ready to be turned off.
4. Click "OK" to shut down the software.
5. Turn off the ViroCyt using the switch on the front of the device.
6. Turn off the air supply and disconnect the air from the inlet.

Troubleshooting

A. CVF Step Failure

1. Remove the CVF vial and insert the SSR vial.
2. Click "Full Wash."
3. Once the full wash is complete, insert the ISW vial and click "ISW."

4. Once ISW is complete, insert the CVF vial and repeat the CVF.
5. Repeat steps A.1–A.4 until the CVF passes.

B. PVS Step Failure

1. Remove the PVS vial, insert the ISW vial, and click “ISW.”
2. Once the ISW is complete, select “Help,” then click on “Prime Syringe.”
3. While the machine enters priming mode, add 10 mL of 70% IPA to two 20 mL luer lock syringes.
4. Remove the yellow cover from the side of the ViroCyt.
5. Attach the syringes via luer ports and adjust the three-way valves so the liquid will move through the system from wash buffer to waste buffer.
6. Slowly depress one of the syringes so the 70% IPA moves through the tubing to the other syringe. This should remove any air bubbles in the system.
7. Once the first syringe is nearly empty (~3 mL remaining), stop depressing it and instead depress the other syringe in the same manner.
8. Repeat steps B.6–B.7 a minimum of 3 times or until all air appears to be removed.
9. Replace the three-way valves for normal operation.
10. Click “Done” on the ViroCyt program.
11. Insert an empty sample vial into the system and click “Reverse Flush.”
12. Once the flush is complete, inspect the vial to ensure it is full of liquid. Dispose of the vial in biohazard trash.
13. Insert the ISW vial and click on “ISW.”
14. Once the ISW complete, remove the vial and insert a fresh PVS vial. Click on “PVS.”
15. If the PVS passes, remove the syringes, empty them into Wescodyne waste, and discard them in biohazard trash. Replace the yellow cover. Continue the main protocol where you left off.
16. If the PVS fails, repeat all of Troubleshooting, B. PVS Step Failure.