SOP: SP078.1
Updated: 7/8/22 KE

Counting cells with EVE Automatic Cell Counter

Materials and Reagents:

1. EVE™ Automatic Cell Counter (VWR Cat# 10027-452)
2. Test Beads for Calibration (ThermoFisher Countess™Test Beads, Cat#C10284, 1mL bottle, $1x10^6$ beads/mL)
3. EVE™ Cell Counting Slides with 0.4% trypan blue dye (VWR Cat# 10027-446, Pack of 50)
4. 0.65 mL Eppendorf tubes
5. 10 µL pipet and filter tips
6. Biohazardous waste bag (to discard tips/tubes)
7. Biohazardous waste sharps container (to discard slides)

Protocol:

1. _____ Re-suspend cell culture well and remove a small aliquot (~100-500 µL) for counting (in 0.65 mL tube).

2. _____ Calibrate EVE™ Automated Cell Counter every time before use (Notes 1 & 2).

3. _____ In a tube, mix 10 µL of aliquoted cell suspension (***mix cell suspension aliquot well beforehand***) and 10 µL of the 0.4% trypan blue dye (***vortex dye beforehand***) (Notes 3 & 4). *This is referred to as a 1:1 solution*.

4. _____ Fill one side of the chamber of the EVE™ cell counting slide with the stained cell suspension. To do so, take 10 µL from the stained cell suspension and slowly dispense it in the sample introduction point (divot in the slide), allowing that the chamber be filled by capillary action.

5. _____ Push slide into slot on the cell counter (Note 5).

6. _____ Adjust focus manually with the green knob (Note 6). Use the "Zoom" feature to check focus for a few sections of the screen.

7. _____ After focusing, press "Count Cells." The counter will automatically count live and dead cells (cells/mL), viability (%), and total cell count (cells/mL) (Notes 7-9).

8. _____ Remove the slide from the counter and write down cell counts. Dispose of slides in a biohazardous waste sharps container.

9. _____ When ready for the next sample count, repeat steps 3-5 then press "Next Sample" on the instrument.

Notes:

1. The **first** step for calibrating the EVE™ counter: use the appropriate media/buffer that the cell suspension is in. Make a 1:1 solution of *appropriate media/buffer: trypan blue dye* for calibration. Add solution to counting slides. The manual provides instructions on how to get to the appropriate calibration buttons on the instrument. ***Calibration of the system should be performed every time before use to set the "background" level for the media/buffer being used.***
2. The **second** step for calibrating the EVE™ counter: use the Countess™Test Beads to check instrument accuracy after manual calibration. Vortex the test beads and make a 1:1 solution of *test beads: trypan blue dye*. Add solution to counting slides. Take an average of two counts with the test beads to ensure accuracy of the instrument.
3. Trypan blue is very toxic and potentially carcinogenic. Always wear gloves, and a lab coat and goggles. Anything that comes in contact with the dye must go in biohazard trash. The EVE™ counting slides can be discarded in a biohazardous sharps containers.
4. If cell suspension is left for an extended period of time in the trypan blue dye, live cells or semi-alive cells will also take up the dye.

5.

Image: https://www.novolab-labware.com/eve-glaasjes-voor-automatische-celteller.html

6. The focus should show live cells and dead cells. Live cells should have clear/bright centers and have a dark halo around them. Dead cells will be blue with no bright centers[1]. If multiple cell counts are needed, one can lock the focus in place with the little black knob near the green knob.
7. The EVE™ Automatic Cell Counter by default calculates for the 1:1 working ***cell suspension: trypan blue dye*** dilutions (i.e. you don't have to count this as a dilution factor in the final count).
8. The **live cell and *total* live cell counts** are important for continual growth (splitting, feeding, etc.) and freezing of cells. Make sure to note any additional dilutions (beyond the 1:1 solution with the dye) made from the cell suspension aliquot. Make sure to note the total cell culture volume (that the cell suspension aliquot was taken from). Below are some important calculations:
   (Live cell count/Total cell count) x 100 = % viability

   Total cell count (cells/mL) x Total volume of cell culture (mL) = total cell count (cells)

   Live cell count (cells/mL) x Total volume (mL) = total LIVE cell count (cells)
9. It is best to run two rounds of counting for the cell suspension. These counts are then averaged. This ensures a more accurate cell density/count (cells/mL). *Make sure to re-suspend the cell suspension well for another count.*

References:

1. EVE™ Automatic Cell Counter. NanoEnTek. May 2014.
   - Website → click "Manual": http://www.nanoentek.com/theme/nanont2_en/shop/02/product01_view.php?it_id=1547538993