Cell enumeration using a microchannel device

PROTOCOL FOR:
Quantification of small cell numbers with a microchannel device

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LEGEND

⇨ ***ATTENTION***

* ***HINT***

REAGENTS

Cell sample to be counted
Microchannel device (1 µl volume, see Reference 1)
Isotonic diluent, such as PBS or saline (Sigma Aldrich, St. Louis, MO, USA)
0.4% Trypan Blue (optional; Sigma Aldrich)

PROCEDURE

1. Isolate cells.
    a. Isolate single cells by fluorescence or magnetic cell sorting
    b. Or remove cells from culture by trypsinization or other methods
    c. Or harvest single cells from primary tissue of interest

2. Dilute/resuspend a small volume of sample cells in isotonic diluent.

3. For viability assessment (optional):
   Dilute cell sample 1:2 with 0.4% Trypan blue solution.

4. Add 1 µl diluted cell sample to microcounter device by pipeting directly into one port.

⇨ Pipette cells into one port only. If the cell suspension does not reach the second port, the suspension may be drawn through the device by inserting pipet tip into second port and pipeting out the remaining air.

5. Count all cells in device using phase-contrast microscopy.

   * Since the sample size is small (i.e., 1–1,000 cells in 1 µl volume), all of the cells in the sample can easily be counted. This will most likely result in the improved accuracy and precision observed for the microcounter compared to a hemacytometer where only a representative field is measured.

6. Calculate cell concentration.

   Cell concentration (cells/µl) = cells counted × dilution factor

EQUIPMENT

Compound light microscope with phase-contrast optics

REFERENCES

1. **Wu, H., T.W. Odom, D.T. Chiu, and G.M. Whitesides.** 2003. Fabrication of complex three-dimensional microchannel systems in PDMS. J. Am. Chem. Soc. *125*:554-559.