Application Note
PBMCs

Concentration & Viability of PBMCs without Lysing

Cellometer®
Cell Viability

Importance of Accurate PBMC Counts

Peripheral blood mononuclear cells are routinely used to measure immunological function through assays for cell proliferation, cytotoxicity, and cytokine expression. The use of cryopreserved PBMCs is necessary in clinical trials involving many patient samples collected over a period of time and even smaller research studies involving multiple time-points.

Ficoll separation is routinely used to isolate mononuclear cells from bone marrow, peripheral blood, and umbilical cord blood. Monocytes and lymphocytes form a buffy coat under a layer of plasma, where the cells are collected and washed. Typically, some residual platelets or red blood cells are mixed in with the mononuclear cells, which cause the separation quality to vary depending upon the patient sample and the operator.

Accurate PBMC cell concentration and viability data should be considered when evaluating results for functional assays. All samples should be evaluated and viability thresholds should be used in clinical trials in order to obtain reliable results.

Introduction: Cellometer Image-based Analysis of PBMCs

The Cellometer Auto 2000 and Vision instruments combine image-based cell counting and dual-fluorescence detection to accurately determine PBMC concentration and viability in heterogeneous samples. The Cellometer Auto 2000 Cell Counter is an all-in-one instrument with built-in touch screen and pre-defined assay selections for simple analysis of PBMCs and nucleated cells. The Cellometer Vision Cell Analyzer offers the added features of user-changeable fluorescence optics modules for additional staining / labeling options and enhanced imaging for analysis of hepatocytes, adipocytes and other sophisticated primary cells.

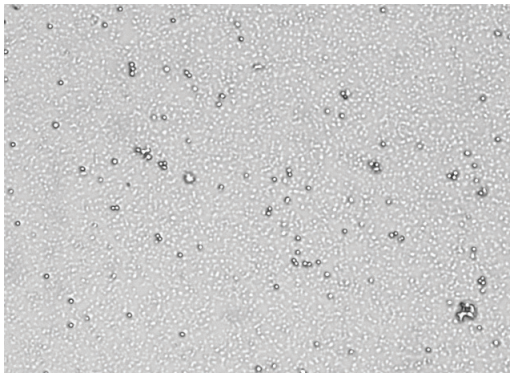
Experimental Procedure

1. Combine 20µl of PBMC sample and 20µl of AO/PI dye mixture and mix well by pipetting up and down
2. Load 20µl of sample into the disposable counting chamber
3. Allow cells to settle in chamber for less than 1 minute
4. Insert chamber into Cellometer instrument
5. Select assay from menu
6. Enter sample ID manually or scan in with bar code reader
7. Preview bright-field cell image and adjust focus (if necessary)
8. Click “Count” to begin analysis
9. Review images and counting results on-screen
10. Images, cell count, concentration, % viability, and cell diameter data are saved to a secure network location

Results

Bright-field Imaging

The Cellometer instrument acquires a bright-field image for each sample tested. The Bright-field image allows researchers to verify cell morphology, evaluate the degree of homogeneity of the sample, and identify the presence of cellular debris.

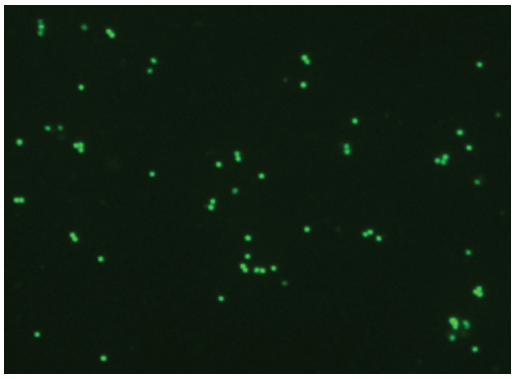


Bright-field image of PBMC sample following Ficoll separation

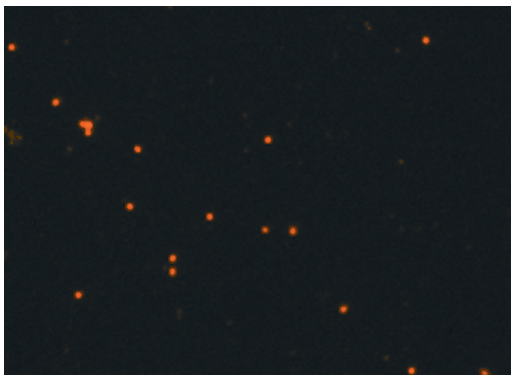
Nexcelom Bioscience

Fluorescent Nucleated Cell Concentration & Viability

Each PBMC sample is incubated with an acridine orange / propidium iodide (AO/PI) dye mixture. The AO dye stains DNA in the cell nucleus of both live and dead cells. Mature mammalian red bloods cells don't contain nuclei, so only peripheral blood mononuclear cells are stained, for a total PBMC count. The PBMC cell count is not affected by platelets, red blood cells, or cellular debris. Propidium iodide DNA-binding dye is used to determine cell viability. Healthy cells are impermeable to the PI dye. Only dead (non-viable) cells with compromised membranes are stained.



Fluorescent image showing nucleated cells stained with acridine orange.



Fluorescent image showing non-viable (dead) cells stained with propidium iodide.

The Cellometer software automatically calculates total PBMC cell count, concentration, and % viability. The PBMC images and data table can be easily saved to a network for additional analysis or data archiving.

Assay: Immune cells, low RBC

Sample ID: new sample
Dilution Factor: 2.00

Count	Concentration	Mean Diameter
Total: 1428 cells	4.93x10^6 cells/mL	6.3 microns
Live: 996 cells	3.44x10^6 cells/mL	6.4 microns
Dead: 432 cells	1.49x10^6 cells/mL	5.9 microns

Viability: 69.8%

Auto 2000 Results Table

Overcome issues related to variability in sample preparation and manual cell counting with automated, dual-fluorescent analysis of PBMC concentration and viability. The Cellometer Auto 2000 Cell Counter and Cellometer Vision Cell Analyzer make it feasible to accurately screen a large number of PBMC samples over time for more accurate interpretation of immunological assay results.

To learn more or request an in-lab demonstration, call 978-327-5340, e-mail info@nexcelom.com, or visit www.nexcelom.com.

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