Technical Note 209

Counting PBMCs in Whole Blood

Introduction

Research settings often require a measurement of peripheral blood mononuclear cells (PBMCs) in a whole blood sample. This information can serve as an indicator of the effectiveness of a treatment or a disease state, for example. Purifying a blood sample can be costly, require a significant time investment, and risks losing critical sample during the multiple cleaning and purification steps. Hand counting with Trypan Blue requires a dilution and purification step to make the sample manageable due to the sheer number of erythrocytes and platelets in the sample. Alternatively, fluorescent counting on the CellDrop™ FL Automated Cell Counter allows a customer to skip the PBMC purification procedure, save timing and money during sample preparation, while increasing count accuracy and reproducibility over manual counting.

Counting Whole Blood with AOPI

The CellDrop uses a dual channel fluorescent system to count whole blood samples. Acridine orange (AO) and propidium iodide (PI) only stain nucleated cells, ignoring extracellular debris and non-nucleated cells. Live cells stained by AO fluoresce green while dead cells fluoresce red when stained PI. The combined dyes can be added to diluted whole blood in a 1:1 ratio, effectively staining the cells of interest.

Preparing Whole Blood for Counting on CellDrop

1. Dilute whole blood sample 1:100 with PBS. Note: Actual dilution factor is sample dependent and should be determined empirically.
2. Combine diluted whole blood with AO/PI in a 1:1 ratio for a final dilution of 1:200.
3. Pipette the sample into the loading groove of the CellDrop with the arm down.
4. Adjust focus/exposure and check the protocol to ensure the correct parameters are selected, press count.

Recommended Whole Blood Protocol

CellDrop count algorithms use multiple parameters, together called a count protocol, to allow a user to fine tune what is counted as a cell. Although some adjustments may still be required, the recommended settings for counting PBMCs in whole blood are presented in Table 1.

Table 1: Recommended Protocol Settings for Whole Blood

Count Application	AO/PI
Chamber Height	100 µm
Dilution Factor	200*
Diameter(min)	4 µm
Diameter (max)	15 µm
Live Roundness	1
Dead Roundness	1
Green Fluorescence Threshold	1
Red Fluorescence Threshold	1

** Adjust to reflect the actual dilution as required*

Ensuring Reproducible Results

The following tips will help ensure that the data from whole blood counts on the CellDrop is consistent and accurate:

- Clean the sample surfaces by loading 15 µl of 70% ethanol before and after each counting session.
- Store AO/PI in the dark at 4°C to prolong the potency of the dyes.
- Thoroughly mix the sample in the tube before loading.
- Check the focus and fluorescent exposure settings before counts and adjust if necessary.
- View the counted image after measurement and adjust parameters if necessary.

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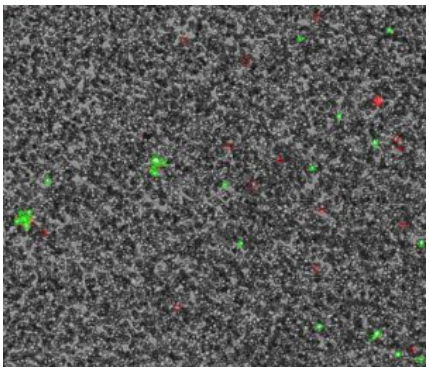

Figure 1: CellDrop FL identifies and counts the live and dead nucleated cells in a whole blood sample.

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