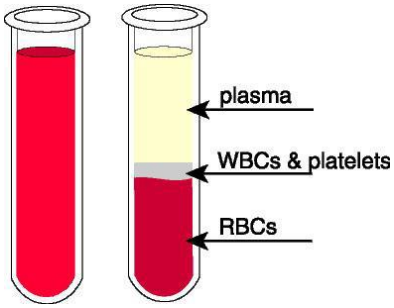
AO/PI Dual Fluorescence Analyzing the Concentration and Viability of PBMC

Introduction

Peripheral blood mononuclear cells (PBMCs) are often processed to separate from whole blood by density gradient centrifugation. Those cells are consist of lymphocytes (T cells, B cells, NK cells) and monocytes, commonly used in the field of immunology, cell therapy, infectious disease and vaccine development. Monitoring and analyzing viability and concentration of PBMC is crucial for the clinical laboratories, basic medical science research and immune cell production.



Isolated PBMC from fresh blood with Density gradient centrifugation

AOPI Dual-fluoresces counting is the assay type used for detecting cell concentration and viability. The solution is combination of acridine orange (the green-fluorescent nucleic acid stain) and propidium iodide (the red-fluorescent nucleic acid stain). Propidium iodide (PI) is a membrane exclusion dye that only enters cells with compromised membranes, while acridine orange is able to penetrate all cells in a population. When both dyes are present in the nucleus, propidium iodide causes a reduction in acridine orange fluorescence by fluorescence resonance energy transfer (FRET). As a result, nucleated cells with intact membranes stain fluorescent green and are counted as live, whereas nucleated cells with compromised membranes only stain fluorescent red and are counted as dead when using the Countstar® Rigel system. Non-nucleated material such as red blood cells, platelets and debris do not fluoresce and are ignored by the Countstar® Rigel software.

Materials and instruments：

1. 100µl suspension PBMC medium
2. 100 ml PBS
3. AO/PI Kit
4. Countstar Chamber slide
5. Countstar Rigel;

Experimental Procedure:

1. Dilute the PBMC sample into 5 different concentrations with PBS;
2. Add 12µl AO/PI solution into 12µl sample, gently mixed with pipette;
3. Draw 20µl mixture into chamber slide;
4. Allow the cells to settle in the chamber for around 1 minute;

5. Insect the slide into Countstar Rigel instrument;
6. Choose the “AO/PI Viability” assay, then test by Countstar Rigel.

Caution: AO and PI is a potential carcinogen. It is recommended that the operator wear personal protective equipment (PPE) to avoid directly contact with skin and eyes.

Result:

1. Bright Field and Fluorescence images of the PBMC

The AO and PI dye are both stains DNA in the cell nucleus of cells. Therefore, Platelets, red blood cells, or cellular debris are unable to affect PBMCs concentration and viability result. Living cells, dead cells and debris can be easily distinguished base on the images generated by Countstar FL (Figure 1).

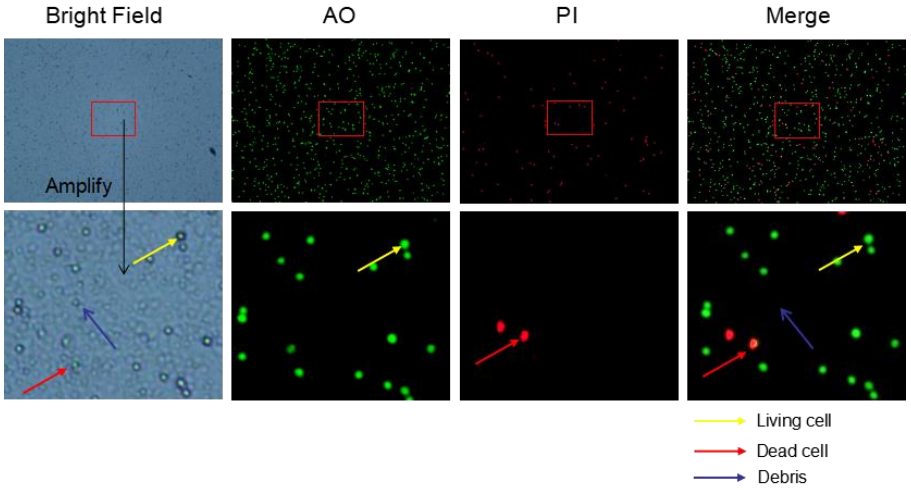


Figure 1. Bright Field and Fluorescence images of the PBMC

2. Concentration and Viability of PBMC

The PBMC samples was diluted in 2, 4, 8 and 16 times with PBS, then those samples were incubated with an AO/PI dye mixture and analyzed by Countstar Rigel respectively. The result of concentration and viability of PBMC is showed as below figure:

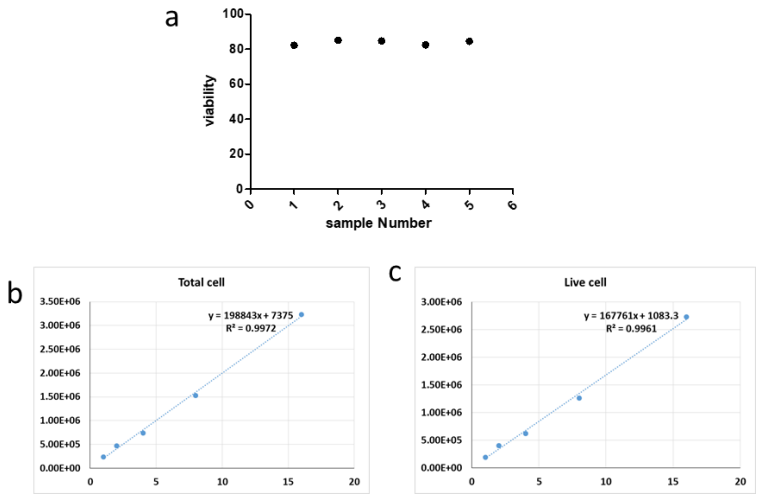


Figure 2. Viability and Concentration of PBMC in five different sample. (a). The viability distribution of

different samples. (b) The linear relationship of total cell concentration between different samples. (c) The linear relationship of live cell concentration between different samples.

Conclusion

Countstar Rigel is able to deliver high resolution image with additional morphological information . And AO/PI fluorescent dye staining method will eliminate the interference of cell debris and avoid miscounts by nucleus-free cell (Fig. 1). The variations of different serial dilution samples viability measured by Countstar Rigel is stable as coefficient of variance is lower than 5 %.(Fig. 2a). The result of concentration of total cells and live cells measured by Countstar Rigel both show a significantly high linear relationship, $R^2 > 0.99$, between 0.2million/ml to 2.73million/ml (Fig, 2b and 2c).