

CD Marker Analysis of T cell by Countstar Rigel System

1. Introduction

CD marker analysis is a typical experiment performed in cell related research fields to diagnose various diseases (autoimmune disease, immunodeficiency disease, tumor diagnosis, haemostasis, allergic diseases, and many more) and disease pathology. It is also be used to test the cell quality in various cell diseases research. Flow cytometry and fluorescence microscope are the routine analysis methods in cell diseases research institutes used for the immuno-phenotyping. But these analysis methods can either provide images or data series, only, which may not meet the strict approval requirements of the regulatory authorities.

The Countstar® Rigel provides a combined solution for the CD marker analysis. Images combined with statistical highly accurate results, the Countstar® Rigel realizes both by one single click.



Figure 1 Countstar® Rigel system combines the functionalities of a digital microscope, an image cytometer and a cell counter in a single bench top instrument.

2. Instruments and Materials

- (1) Expand T cells from nasopharyngeal carcinoma tissue;
- (2) CD45-FITC, CD4-Percp, CD8-PE antibody, supplied by Ruiyu Company;
- (3) Chamber slide, supplied by Ruiyu Company;
- (4) Countstar Rigel

3. Method

- (1) Prepare 0.5 million cells for test, wash with PBS for one time.
- (2) Add 100ul PBS, then add 5µl CD45-FITC, CD4-Percp, CD8-PE antibody respectively, incubate in dark for 20 minutes.
- (3) Wash with PBS for one time, then suspended with 100µl staining buffer.
- (4) Draw 20µl mixture into chamber slide.
- (5) Allow the cells to settle in the chamber for around 1 minute, then test by Countstar Rigel.

4. Result

4.1 Images of T cell Analyzed with Countstar Rigel

Three signal-color application procedure was created by setting Green, Red and Yellow channel to fluorescent images of CD45-FITC, CD4-Percp, CD8-PE, plus a bright field. Bright field picture reference segmentation was applied as a mask to sample the fluorescence signal. Example images of T cells are shown in Figure 1.

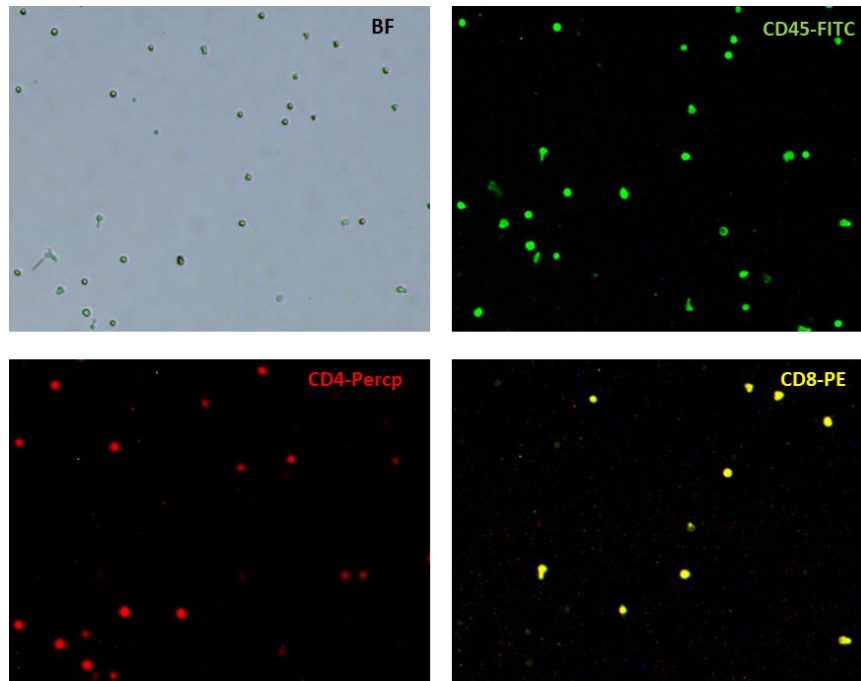
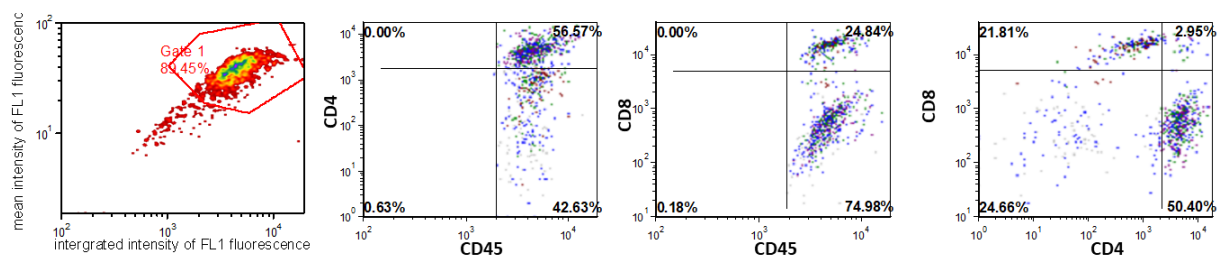


Figure 2 The representative images captured by Countstar Rigel (fractionated gain, not a full image).
Upper left: Bright field, upper right: Ex: 480nm, Em: 535nm, down left: Ex: 480nm, Em: 665LP, down right: Ex: 480nm, Em: 580nm.

4.2 Quantify the percentage of T cell with different CD Marker by FCS express

Use FCS express to analysis the data like flow cytometry, gating the cells dependent on the cell population and fluorescence intensity (Figure 2). The percentage of T cells with CD45+CD4, CD45+CD8 and CD4+CD8 is presented in the Table 1.



	CD45+CD4+%	CD45+CD8+%	CD4+CD8+
Sample	53.34%	25.81%	2.06

Figure 3 The result of sample1 was analyzed by FCS software, the first figure from left is gating the live cell for further analysis. Table1: The statistical results were shown in the table1.

5. Conclusion

The Countstar Rigel system provides a rapid and easy means of evaluating CD marker. FCS express supplies the function to review every signal cell, validation the data through the image. The user can also have confidence to carry out next experiments based on Countstar Rigel results.