Quantitative Assessment of Myeloid Nuclear Differentiation Antigen using Flow Cytometry

Authors: Kah Teong Soh, Joseph D. Tario, Jr., Paul K. Wallace

Abstract

Myelodysplastic Syndrome (MDS) is a set of clonal marrow failure disorder that are difficult to diagnose due to the lack of standard diagnostic parameters, admixture of normal bone marrow in MDS sample and large differential diagnosis in MDS. A combination of markers must be used due to the absence of single marker that can reliably distinguish MDS from non-MDS patient. The ultimate goal of this study is to design the best cocktail that could be applied to diagnose the disease with high confidence, sensitivity and specificity. Cell were lysed and permeabilized for both intracellular and intracellular staining and the intensity of the signals were assessed using flow cytometry. A standard protocol based on formaldehyde & saponin (CalTag) and one developed by Chow et al., which based on formaldehyde & Triton X-100 were compared to determine which methodology provides better permeabilization as it is crucial to our intracellular staining. Chow et al. based methodology was deemed to be superior in permeabilizing cell; both THP1 and U937 cells lines produced positive signals. However, cell lines K562 did not provide enough information, of whether the signals came from intracellular staining, or from the remains of anti-MNDA stain that stayed in the cells. An isotype was used and it was found that K562 were stained positive by anti-MNDA, even though the signal was approximately 3 to 5 times weaker compared to THP-1 and U937. The exclusion of methanol from the washing step did not weaken our fluorescence signals.

Materials and Methods

Treatment with and without Methanol

<table>
<thead>
<tr>
<th>Tubes</th>
<th>Permeabilizing Agent</th>
<th>Wash with Methanol?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Set 1</td>
<td>CalTag</td>
<td>No</td>
</tr>
<tr>
<td>Set 2</td>
<td>10% Formaldehyde</td>
<td>No</td>
</tr>
<tr>
<td>Set 3</td>
<td>10% Formaldehyde</td>
<td>yes</td>
</tr>
</tbody>
</table>

Staining, Fixation, and Permeabilization

- 100 µL of cells were transferred into tubes. Tubes labeled with CD45 were stained with CD45 – APC
- Mouse anti-IgG was added to the tube. Incubation was done on ice for 30 minutes followed by washing steps.
- For tube set 2 and set 3, 65µl 10% formaldehyde was added, followed by 1 mL of Triton X-100. For set 1, 2 mL of CalTag was added. Samples were incubated for 30 minutes at room temperature.
- 1mL cold buffer consist of 4% fetal bovine serum diluted in PBS was added into each tube followed by centrifugation. Tube s&2 and tube 3 were resuspended using 1mL PBS and 1mL 50% methanol respectively.
- Tube labeled with MNDA were stained with anti-MNDA and incubated at room temperature for 30 minutes. Centrifugation was performed and the cell pellets were resuspended to 2mL.
- Emission spectrum was acquired using LSR-II B flow cytometer. Data analysis was performed using WinList 7.0

Result

Figure 2: Control (A) compared to CD 45 and MNDA staining (B). Tests were done in triplicate (Table 1). Both U937 and THP-1 cell lines illustrate positive staining for MNDA and CD 45.

Table 2: Measurement of mean and standard deviation of triplicate using K562 cell lines. Isotype was detected as a negative signal. Therefore, the intracellular staining of K562 using anti-MNDA illustrated true positive.

Table 1: Measurement of mean and standard deviation of triplicates (data not shown) using U937 and THP-1 cell lines. Both cell lines showed positive binding to surface marker and intracellular stain.

Citation of Literature