Viability / 7-AAD

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Overview

This protocol describes the procedure for performing viability assessment of substrates submitted for surface marker phenotyping using flow cytometry. The advantage of this technique, in comparison to the "classic" non-cytometric trypan blue exclusion method, is that the viability of a specific bit-mapped cell population can be determined. An added advantage is the capability of performing the viability assessment within the context of expression of surface markers identified with fluorochrome-labelled antibodies. The dye used in this procedure is **7-amino-actinomycin D** (7AAD), which is suitable for dead cell discrimination in lengthy experiments because it is efficiently excluded by intact cells and has a high DNA binding constant. In addition, the dye is valuable in combination with other fluorochromes on a single argon laser instrument, since its emission in the far red can easily be separated from the emission of commonly used FITC and PE conjugates. An example is depicted, demonstrating the difference in viability between lymphocytes and granulocytes. Cells within the lymphocyte bitmap (middle panels) maintain good viability, comparing two hours post-draw (top) and following overnight storage (bottom). In contrast, granulocytes (right panels) begin to deteriorate within two hours (top), and show significant cell death following overnight incubation (bottom).

Specimens requiring viability checks

* all lymph node cell suspensions from Surgical Pathology
* all bone marrow aspirates > 24 hours old
* all peripheral blood specimens > 24 hours old

Reagents

* Via-Probe (Pharmingen)
* aqueous 7-AAD solution, approximate concentration is 0.01% (100 µg/ml, varies from lot-to-lot)
* resulting staining concentration approximates 15-20 µg/ml
* handle with same precautions as 7-AAD
* CD45*FITC/CD14*PE

Methodology

* add 20 µl of Via-Probe and 20 µl of CD45/CD14 to 100 µl cells (@2-5 x 10e6/ml)
* process as for surface marker phenotyping using whole blood lysis (ammonium chloride only, see note below)
* collect a listmode file on the cytometer, using the same protocol as will be used for phenotyping the specimen

* perform off-line listmode analysis with WinList using the "7-AAD" protocol; examine and evaluate the following histograms:
* 7-AAD, **ungated**
1. region 1: viable
2. region 2: putative apoptotic
3. region 3: dead
* CD45 versus LSS (color eventing with regions 1, 2, 3)
1. region 4: adjust bitmap to approximate that used for phenotyping
* 7-AAD, **gated** through region 4
1. region 5: viable
2. region 6: putative apoptotic
3. region 7: dead

* Viability enumeration:
* region 1 represents the viability of the entire sample
* region 5 represents the viability of the population being analyzed
* NOTE: This procedure cannot be used with lysing reagents that contain fixative (eg. DAKO Uti-Lyse, BD FACS-Lyse) since the cell membranes are permeabilized during lysis, and all the cells appear to be either "apoptotic" or dead. All of our stained, fixed cells are analysed within 30 minutes; the reliability of viability determinations of cells that are stained and held overnight prior to analysis is suspect.

References

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* Philpott NJ, et al. The use of 7-Amino-Actinomycin D in identifying apoptosis: Simplicity of use and broad spectrum of application compared with other techniques. *Blood* 87(6), 2244-2251 (1996).

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