Rare Cell Population Detection With StarBright Dye Antibody Conjugates

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Introduction

Rare cell populations, like hematopoietic stem cells (HSCs) and circulating endothelial cells in peripheral blood, are cells with extremely low frequency (usually less than 0.002% of the total population). Although rare, they are important cell subsets for studying and potential biomarkers of disease diagnosis. It is challenging to detect and characterize these cells by flow cytometry, due to their low frequency and complex phenotypes, as well as the limitation of current fluorescent dyes on the market. Many conventional dyes are dim, and tend to aggregate, which can lead to consistency issues and may easily degrade under certain conditions, which are challenges for building consistent multiplex panels, especially for rare cell population detection on flow cytometry.

Here, we demonstrate how new Starbright™ Dye antibody conjugates, with unique features, facilitate the detection and characterization of rare populations. Starbright Dyes are novel fluorescent molecules with superior brightness over conventional dyes. Starbright Dyes also have narrow excitation and emission spectra, in relation to traditional fluorophores. Starbright Dye series (UltraViolet, Violet, Blue, Yellow, and Red) are suitable for all instrumentation with the appropriate lasers and filters in conventional flow cytometry, and have been shown to work in full spectrum flow cytometry. Based on the above traits of Starbright Dyes, their broad range of brightness provides greater flexibility for building multiplex panels. Also, the overall brightness of Starbright Dyes allows for more options for detecting low-abundance antigens in multiplex panels. High stability and low autofluorescence of Starbright Dyes, in membrane treatment (an important cell permeabilization step for cellular protein detection) or staining, offers a new approach for characterizing rare populations. Moreover, we can also detect rare cell subsets — HSCs in human peripheral blood mononuclear cells (PBMCs), with eight-color multiple panels containing Starbright dye antibodies and conventional fluorochrome-conjugated antibodies.

In summary, Starbright Dyes are superior choices for detecting and characterizing rare cell populations, due to their brightness, stability, and consistency.

Materials and Methods

Excitation and Emission Spectra: The Starbright Yellow (SBY) spectra were generated using Bio-Rad’s Spectraviewer available at: https://www.bio-rad.com/.

Staining Conditions for Stain Index Data: Human peripheral blood mononuclear cells (PBMCs) were stained in a 96-well plate for 3 hr at 4°C in the dark with a mouse anti-human CD3 Starbright Dye antibody conjugate, washed three times, and resuspended in PBS Buffer. All antibodies were titrated to determine the optimal staining concentration prior to use.

Data Collection and Analysis: Data for these studies were collected on a 5-laser 35-parameter Bio-Rad BD Cell Analyzer and analyzed using FcSpectra (Becton, Dickinson & Company) and FCS Express (DeNovo).

Stain Index (SI) Calculation: Single cell lymphocytes were gated to identify positive and negative populations on the target channel. The Median Fluorescence Intensity (MFI) of both populations was quantified and the negative population’s robust Standard Deviation (SD) calculated. Stain Index (SI) was calculated using the following formula:

\[ SI = \frac{\text{MFI}_{\text{target}} - \text{MFI}_{\text{background}}}{\text{SD}_{\text{background}}} \]

Meethanol (MeOH) Treatment on Staining Stability: Human peripheral blood mononuclear cells (PBMCs) were stained with anti-CD4 Starbright Yellow (SBY670) and anti-CD4 PE-Dazzle 594 (as control) for 3 hr at 4°C, followed with washes (2× PBS for 30 mins) and methanol treatment (50% cold pure MeOH at 4°C, for 30 mins). Cells were washed two times and resuspended in the FACS buffer prior to data acquisition.

Multiplex panel: PBMCs were stained with antibody dye conjugates, as shown in Table 1. All antibodies were titrated to determine the optimal staining concentration prior to use. Single staining and fluorescence minus one (FMO) controls were set up for compensation and gating. Hematopoietic stem cells (HSCs) were identified as Lin-CD3-CD122-CD135-CD45-CD11b-CD38-CD45RA-CD19-60%

Starbright Dyes Have Narrow Excitation/Emission Spectra and Minimal Spillover

1. Excitation and Emission Spectra of Starbright Yellow (SBY) Dye: Starbright Dyes have narrow excitation and emission spectra. Starbright Dyes have the ability to detect dim signals.

2. Starbright Dye Antibody Conjugates are superior choices for detecting and characterizing rare cell populations.

3. Starbright Dye Brightness (Stain Index) Ranking:

   - StarBright Dye Antibody conjugates are superior choices for detecting and characterizing rare cell populations.

   - Using new StarBright Dye Antibody conjugates, researchers can achieve higher sensitivity and specificity in detecting rare cell populations.

   - The high brightness of StarBright Dye Antibody conjugates allows for more consistent and reliable detection results.

   - The narrow excitation and emission spectra of StarBright Dyes provide better flexibility for building multiplex panels.

   - The stability of StarBright Dyes ensures consistent performance in various staining conditions.

   - The high brightness and narrow excitation spectra of StarBright Dyes facilitate the detection of low-abundance antigens in multiplex panels.

Conclusions

Features of Starbright Dyes:

- Narrow excitation and emission spectra
- Bright
- Highly stable
- Flexible to build multiplex panels for rare cell identification

Starbright Dye Antibody conjugates are superior choices for detecting and characterizing rare cell populations.