SpectraDye™ Antibody Labeling Kit

Convenient antibody labeling technology, for quality flow cytometry data

The SpectraDye™ Antibody Labeling Kit is a convenient, easy-to-use technology to prepare fluorescent antibody conjugates. Create your own fluorescent secondary or label your favorite primary antibody for direct detection.

Direct detection with fluorescent antibodies saves time and increases reproducibility while eliminating non-specific background caused by secondary antibodies. Another added benefit of eliminating secondary reagents from your experiments is that you have the freedom to choose your best primary antibody regardless of species. With six commonly used fluorescent dyes to choose from, SpectraDye™ Antibody Labeling Kits give you tremendous flexibility.

Introduction

Choosing suitable antibodies can be time consuming and expensive since extensive optimization is usually required. Directly labeling your primary cuts optimization time in half, since optimizing a secondary is unnecessary.

In this application note, we demonstrate the utility of the SpectraDye™ Antibody Labeling Kit in generating quality flow cytometry data. In the first experiment, HeLa cells were stained with 3-color antibody panels to demonstrate the ease of multiplexing with SpectraDyes™. In the second experiment, Stem Trol™ cells were stained with SpectraDye™ antibody conjugates and commercially available Alexa Fluor® antibody conjugates to show that the dyes perform comparably.

Primary Antibody	SpectraDye™ used for labeling	Excitation (nm)	Emission (nm)	Product #
GAPDH	Dye 650	653	672	K-11057-010
Actin	Dye 550	551	565	K-11056-010
Tubulin	Dye 490	491	515	K-11055-010
Golgin97	Dye 650	653	672	K-11057-010
COXIV	Dye 550	551	565	K-11056-010
CD34	Dye 650	653	672	K-11057-010
CD45	Dye 490	491	515	K-11055-010

Table 1. Antibodies and the corresponding dyes with which they were labeled prior to use for flow cytometry.

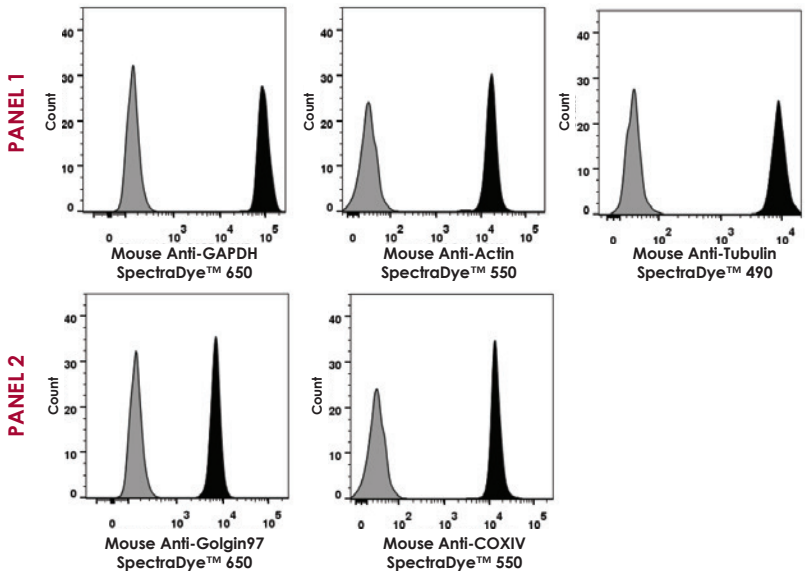

Figure 1. Antibodies labeled with the Spectradye™ labeling kits recognize targets by flow cytometry. Histograms represent stained (black) and unstained (gray) cells in each population.

Results

SpectraDye™-labeled antibodies demonstrate excellent performance in flow cytometry experiments, providing strong, easy-to-analyze signals.

Multiplexing with Spectradye™ primary antibodies

To test the functionality of antibodies labeled with the SpectraDye™ fluorophores, five proteins were chosen as targets of interest: tubulin, actin, GAPDH, COXIV, and Golgin 97. Primary antibodies against these proteins were labeled with the SpectraDye™ fluorophores listed in Table 1. After antibody labeling, HeLa cells were fixed, permeabilized, and then treated with a panel of labeled antibodies, consisting of either Panel I (GAPDH/ Tubulin/ Actin) or Panel 2 (COXIV/ Golgin97/ Actin). All three antibodies in each set were able to stain the HeLa cells. The intensity of staining varies from cell to cell, as is typical in staining procedures. The distribution of labeled cells can be seen in the histograms (Figure 1).

SpectraDye™ labeled Abs perform comparably to commercially available Alexa Fluor® conjugated Abs

The ability to create your own labeled primary antibodies provides convenience, flexibility, and increased reproducibility. To demonstrate that SpectraDye™ labeled antibodies perform comparable to commercially available reagents, antibodies against surface proteins CD34 and CD45 were evaluated. Anti-CD34 and anti-CD45 were labeled with SpectraDye™ 650 and 490, respectively, and tested against the commercially available antibody labeled with Alexa Fluor® equivalents. StemTrol™ control cells (CD34/ CD45 positive) were spiked into SKUT-1B (CD34/CD45 negative) cells that were used as background for staining.

Flow cytometry results with SpectraDye™-labeled antibodies and Alexa Fluor® antibodies showed comparable staining. The CD45 and CD34 positive StemTrol™ cells were similarly stained with both sets of antibodies, as shown in Figure 2. The background SKUT-1B cells did not show non-specific staining with either set of labeled antibodies.

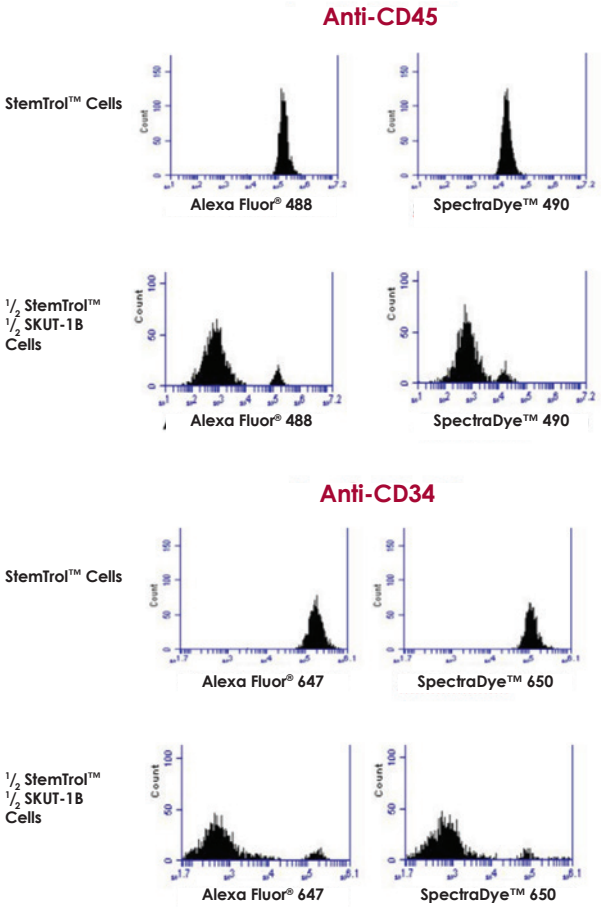

Figure 2. StemTrol™ (CD34/CD45 positive cells) and SKUT-1B cells (CD34/CD45 negative cells) were stained with anti-CD34 and anti-CD45 antibodies labeled with SpectraDyes™. For comparison, commercially available Alexa Fluor® Labeled antibody equivalents were used. Histograms show the distribution of the stained cells.

Conclusions

The SpectraDye™ Antibody Labeling kit is a convenient, rapid, and effective means to generate antibodies for quality flow cytometry experiments. Antibodies labeled with SpectraDye™ can be used to stain intracellular or surface targets. The staining of cells with SpectraDye™ -labeled antibodies is comparable to commercially available Alexa Fluor® conjugates – meaning you now have the ability to perform direct fluorescent detection with any primary antibody.

Methods

Primary antibodies and reagents

Primary antibodies anti-GAPDH, Tubulin, Actin, COXIV, Golgin97, CD34, and CD45 were labeled with SpectraDye™ as listed in Table 1. Commercial antibodies tested included anti-CD34 Alexa Fluor® 647 and anti-CD45 Alexa Fluor® 488.

Antibody labeling

Labeling was performed according to recommended instructions. Briefly, SpectraDye™ Ab Labeling Buffer was added to the antibody solution and mixed. Dye solution was added to the antibody solution, mixed, then incubated at room temperature 30 minutes. Quenching Solution was added, mixed well and incubated at room temperature 5 minutes, followed by Neutralization Buffer.

Growing and preparing cells for flow cytometry

HeLa cells were grown for 2 days in DMEM (with 10% FBS, 1 % Pen/Strep). Cells were pelleted and fixed in 4 % paraformaldehyde. Cells were pelleted again and resuspended in PBS with 0.1 % triton for 5 min to permeabilize the cells. Cells were pelleted and resuspended in PBS. SpectraDye™ Antibodies were added to the cells at 330 µg/mL and incubated 30 minutes. Cells were then washed with PBS twice and pelleted. Cells were resuspended in PBS for flow cytometry.

SKUT-1B cells (CD34/CD45 negative cells) were grown to log phase, then trypsinized to collect single cells. SKUT-1B or StemTrol™ cells (CD34/CD45 positive cells) were resuspended in incubation buffer (PBS containing 2% FCS, 2 mmol/L EDTA, and 0.1% (wt/vol) NaN3) at 1x106 cells/ml and then aliquoted into the appropriate sample tubes. Cells were washed once with incubation buffer and pelleted, followed by resuspension in incubation buffer. SpectraDye™ labeled antibodies and commercial Alexa Fluor® labeled antibodies were added at 200 µg/mL per tube, followed by 1 hour incubation. After incubation, cells were washed twice with incubation buffer before preparing for flow cytometry.

Flow Cytometry

For the HeLa cells, flow cytometry was performed with an LSRFortessa 14-color cytometer. Compensation was performed for APC and PE channels. For the second experiment, SKUT-1B and StemTrol™ cells were analyzed on the Acurri C6 flow cytometer. Data was analyzed with with FlowJo software (TreeStar).

	Excitation maximum (nm)	Emission maximum (nm)
SpectraDye Antibody Labeling Kit-350	353	432
SpectraDye Antibody Labeling Kit-490	491	515
SpectraDye Antibody Labeling Kit-550	551	565
SpectraDye Antibody Labeling Kit-650	653	672
SpectraDye Antibody Labeling Kit-IR700	690	709
SpectraDye Antibody Labeling Kit-IR800	783	800

Ordering Information

Catalog Number	Product	Size	Each SpectraDye Antibody Labeling kit includes the following
K-11054-010	SpectraDye™ Antibody Labeling Kit-350	1 kit	• Antibody Labeling Buffer • SpectraDye Dye Solution* • Quenching Solution • Neutralization Buffer Each kit includes sufficient reagents to label up to 1 mg of antibody
K-11055-010	SpectraDye™ Antibody Labeling Kit-490	1 kit	
K-11056-010	SpectraDye™ Antibody Labeling Kit-550	1 kit	
K-11057-010	SpectraDye™ Antibody Labeling Kit-650	1 kit	
K-11058-010	SpectraDye™ Antibody Labeling Kit-IR700	1 kit	
K-11059-010	SpectraDye™ Antibody Labeling Kit-IR800	1 kit	
K-11060-010	SpectraDye™ Antibody Labeling Kit, user-supplied dye	1 kit	

* Kit K-11060-010 (user-supplied dye) does not include SpectraDye Dye Solution

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