

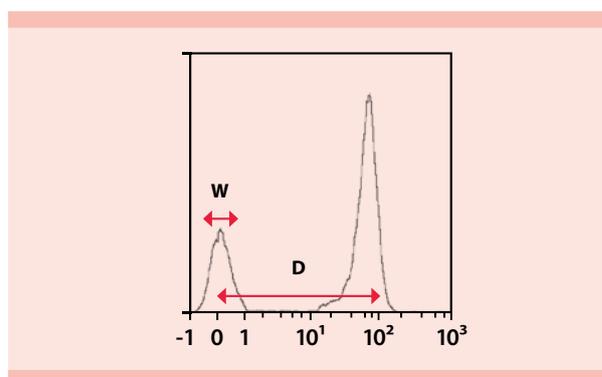
Find the brightest option for your flow cytometry experiments

# Fluorochrome brightness index

Flow cytometry has an inherent need to continuously increase the number of fluorochromes to meet the growing demand of sophisticated multicolor analysis. However, fluorochromes vary greatly with regard to their fluorescence intensity. In particular for antigens with low expression levels, it is crucial to select an appropriate fluorochrome-conjugated antibody to obtain sufficiently bright signals for reliable analysis.

In flow cytometric analysis, fluorescence intensity for individual conjugates can be evaluated (table 1) according to the stain index (SI) and the mean fluorescence intensity (MFI). SI is a measurement that takes into account both the distance (D) between the positive and background peaks and the spread (W) of the background peak (fig. 1). SI is defined by the quotient of D/W. MFI defines the mean intensity of the fluorescence measured in a specific channel. MFI does not take into account the spread of the background peak, only the brightness of the positive peak.

SI and MFI values can differ due to a number of variables, including antigen target and antibody clone, the flow cytometer's filters, settings, and laser power, as well as buffer conditions. Therefore, the evaluation in table 1 should be regarded only as an indicator of brightness. In all cases, the individual product pages on our website, including example histograms or dot plots, should be consulted for details on particular fluorochrome-conjugated antibodies. Comprehensive information is available at [www.miltenyibiotec.com/antibodies](http://www.miltenyibiotec.com/antibodies)



**Figure 1:** SI is defined by the quotient of D/W, whereas MFI is defined only by the brightness of the positive peak.

Fluorochrome	Brightness based on MFI	Fluorochrome	Brightness based on SI
VioBright™ 515	5	VioBright 515	5
APC	5	APC	5
VioBright FITC	5	VioBright FITC	5
PE-Vio® 770	5	PE	5
PE	4	PE-Vio 615	5
PE-Vio 615	4	PE-Vio 770	4
APC-Vio 770	3	FITC	3
VioBlue®	3	Vio 515	3
Vio 515	3	PerCP-Vio 700	3
PerCP-Vio 700	2	APC-Vio 770	2
FITC	2	VioBlue	2
VioGreen™	1	PerCP	1
PerCP	1	VioGreen	1

**Table 1:** Data was generated using the MACSQuant® Analyzer 10. Human peripheral blood mononuclear cells were stained with CD4 (VIT4) antibodies conjugated to the indicated fluorochromes. Stain indices and MFI values may vary depending on instrument, instrument configuration, reagents, and cell type used (6 = brightest, 1=dim).

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