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BioLegend communication regarding unusual fluorescent signal in a subset of donor samples

When performing cell analysis, donor-to-donor variation in the signal detected is commonly observed when using antibody-mediated techniques such as flow cytometry and microscopy. In certain outlier cases, donors display an aberrant signal on specific cell subsets. Beginning in early 2021, without any change to our relevant products, we have observed an increase in the number of donors showing unexpected signals with certain antibody conjugates amongst COVID-19 vaccinated donors.

The cases we have observed are limited to workflows where antibody staining is performed in whole blood, using fluorophore antibody conjugates incorporating PEG. Preliminary data indicates a correlation between this unusual signal and high levels of anti-PEG antibodies (data not shown).

The intensity of PEG-related signal in these individuals may depend on the titer of anti-PEG antibodies in serum and the amount of PEG present in the conjugate, among other factors. Table 1 includes a list of BioLegend antibody-conjugates containing fluorophores that may be PEGylated to some degree. Thus, if you are using conjugates incorporating PEG, such as those reagents shown in Table 1, we are providing guidance based on our current experimental results.

If you observe staining patterns similar to those described below (Figures 1 and 2), we recommend the following protocol modification.

Wash the whole blood sample twice with at least 4 times the sample volume using cell staining buffer (BioLegend Cat# 420201) or equivalent. Brief protocol:

a. Add cell staining buffer to bulk blood
b. Centrifuge at 350xg for 5 minutes at room temperature.
c. Aspirate liquid. Whole blood does not make a solid pellet after washing. Please aspirate enough volume to leave approximately the initial sample volume. Example: if washing 1 ml of blood, add 4 ml of cell staining buffer, centrifuge, aspirate 4 mL, discard aspirate
d. Repeat steps a - c
e. Aliquot sample for staining

We have noticed that in most cases the aberrant staining can be blocked by the addition of PEG to the staining buffer (Fig. 2).

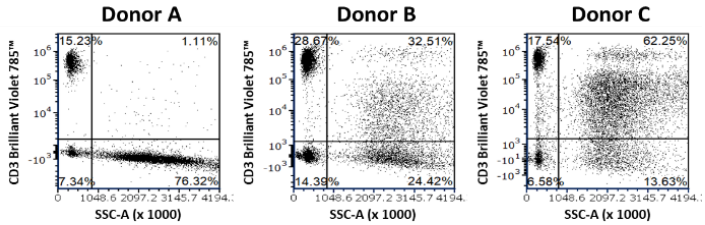

Fig 1. Donor variation and levels of aberrant signal. Whole blood was stained with anti-human CD3 Brilliant Violet 785™ using a lyse wash blood protocol. Donor A – expected pattern. Donors B and C – varying degrees of non-specific staining observed primarily on granulocytes.

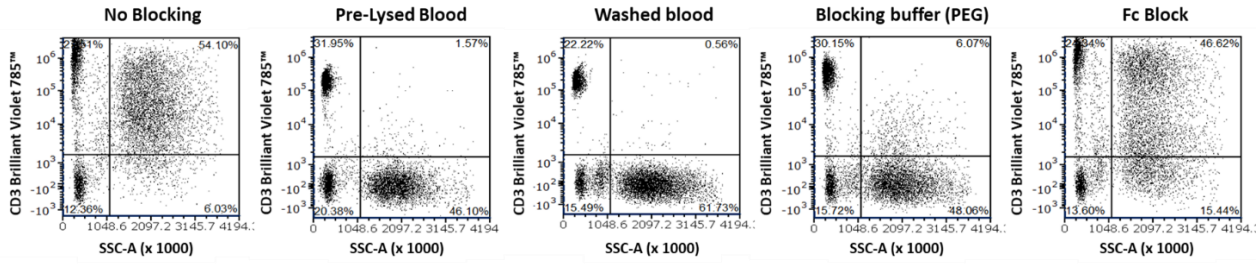

Fig 2. Non-specific staining can be blocked by pre-washing or adding PEG-containing buffer, but not using Human TruStain FcX™ (Fc Receptor Blocking Solution) (BioLegend Cat#422301/2). Samples from the same donor were stained with anti-human CD3 conjugated to Brilliant Violet 785™ and treated as indicated. Dot plots are gated on the entire leukocyte population. Up to 10% of positive granulocyte signal can be considered within an expected range.

Table 1: List of BioLegend antibody conjugates known to contain PEG.

Brilliant Violet 421™	Brilliant Violet 510™	Brilliant Violet 570™	Brilliant Violet 605™
Brilliant Violet 650™	Brilliant Violet 711™	Brilliant Violet 750™	Brilliant Violet 785™
APC/Fire™ 810	PE/Fire™ 700	Spark Violet™ 538	Spark Blue™ 550
Spark NIR™ 685	KIRAVIA Blue 520™		

Please contact our technical service department if you have further questions.
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