Flow Post-its
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The Impact of Washes on Staining

December 2020

In flow cytometry experiments, **sample preparation is** a **key** component for having good quality data. The washing steps in a staining protocol allow for the removal of excess antibody. Single washing protocols may not be sufficient to remove this unbound antibody, which can negatively impact the Stain Index.

$$Stain\ Index = \frac{median_{pos} - median_{neg}}{2\sigma_{neg}}$$

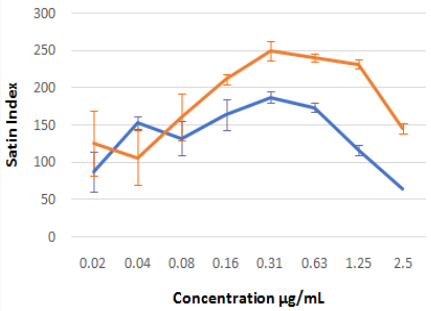

Fig.1 Stain index graph comparison between one and two washes in different PE-CF594-CD4 concentrations (N=3).

The Stain Index is higher for samples that have been washed twice compared to those washed only once.

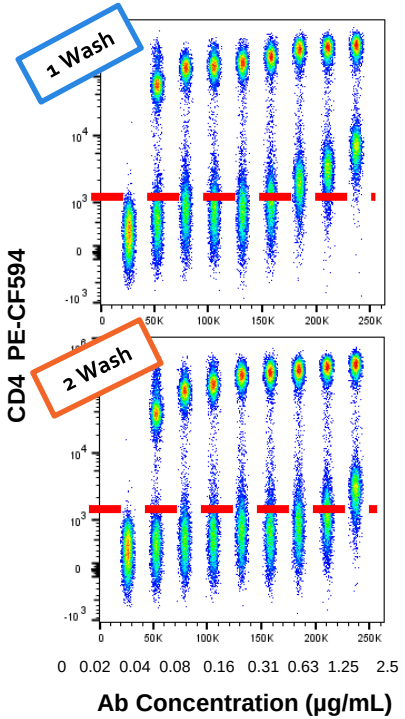

Fig.2 Graph representation between one and two washes in different PE-CF594-CD4 concentrations (N=3).

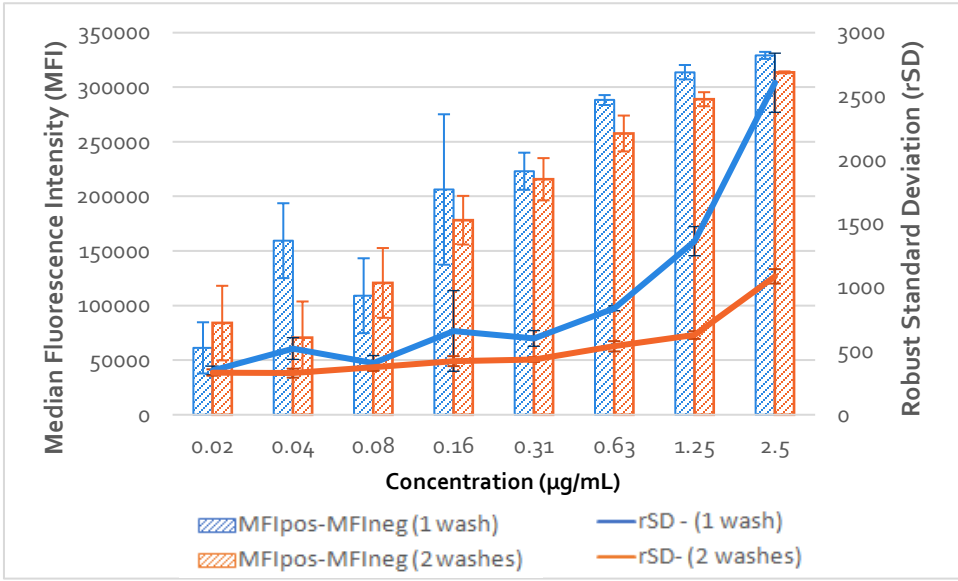

Fig.3 Graph representation of MFIpos-MFIneg and rSD between one and two washes in different PE-CF594-CD4 concentrations (N=3).

Experiment above shows in detail the comparison of an antibody titration of CD4 PE-CF594 stained lymphocytes that were washed either one or two times. While the difference between positive and negative Median Fluorescent Intensity (**MFI**) does not show dramatic changes in the comparison, the Robust Standard Deviation (**rSD**) of the negative population is significantly lower when the samples were washed twice, resulting in a higher Stain Index. **Including more washing steps in the staining protocol can improve resolution of populations and result in better quality data**.