Antigen Density for Human and Murine Surface Markers

Quick Guide

The number of antigens, or target molecules that a cell carries, directly correlates to the intensity of the positive population and will determine the optimal fluorophore you should use for each marker.

Pairing dim fluorophores with highly expressed antigens and bright fluorophores with low abundance antigens will improve the resolution of cell populations, but how do you measure the density?

This guide lists the antigen density of some common human and murine markers to help you choose the right fluorophore and provides step-by-step instructions on how to measure antigen density using antibody binding beads.

Fluorophore Pairing

For the best results, low density antigens should be paired with bright fluorophores and high density antigens with dimmer fluorophores.

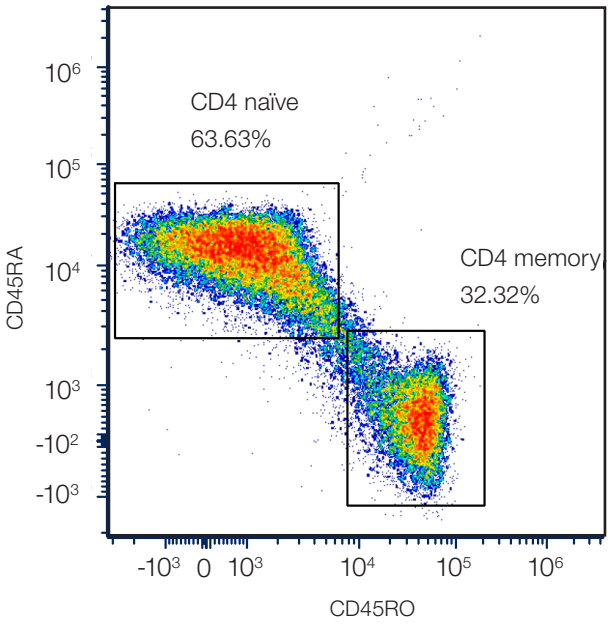

Fig. 1. Staining example. Human peripheral blood lymphocytes were stained with CD45RA A700 and CD45RO SBB700. Data acquired on the ZE5 Cell Analyzer.

Fluorophore Brightness

This example illustrates how, by pairing CD4 with a bright fluorophore (PE), the resolution can be improved.

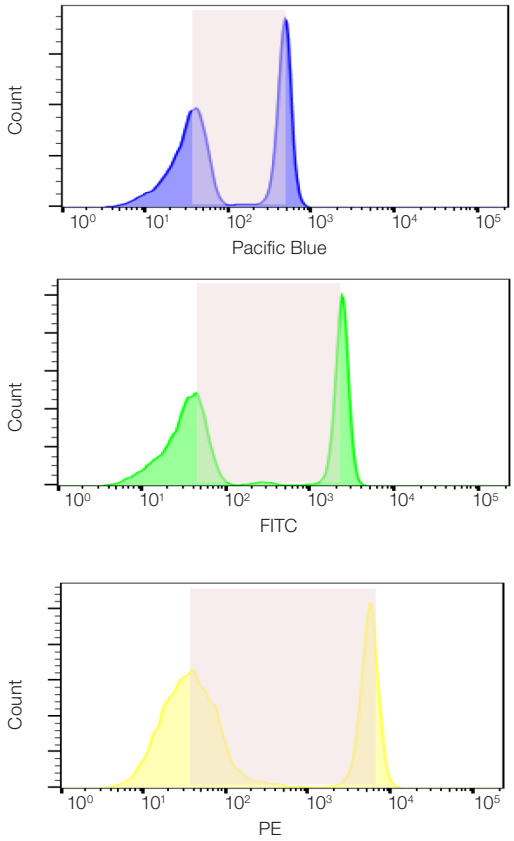

Fig. 2. Fluorophore relative brightness. Examples of CD4 staining of peripheral blood showing the relative brightness of three fluorophores from dim (Pacific Blue) to bright (PE). Data shown was acquired on the ZE5 Cell Analyzer.

Visit bio-rad-antibodies.com/antigen-density for more information.

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Antigen Density for Common Murine Markers

Antigen density of common murine markers found on the surface of freshly isolated splenocytes from C57BL/6 mice.

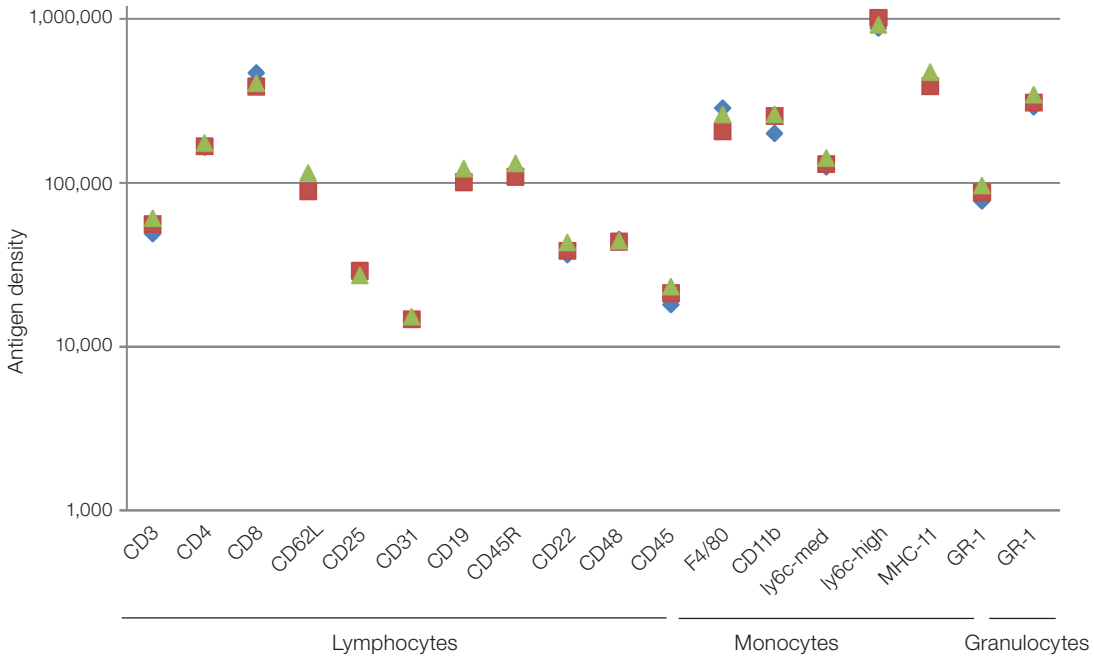


Fig. 3. Data from 16 common murine markers. Geometric mean fluorescence intensity was obtained and converted into antigen density for each surface marker and plotted on a log scale. The results for three independent experiments on C57Bl/6 mice are shown. Data acquired on the ZE5 Cell Analyzer and analyzed in FCS Express software.

Antigen Density for Common Human Markers

Antigen density of common human markers found on the surface of freshly isolated peripheral blood.

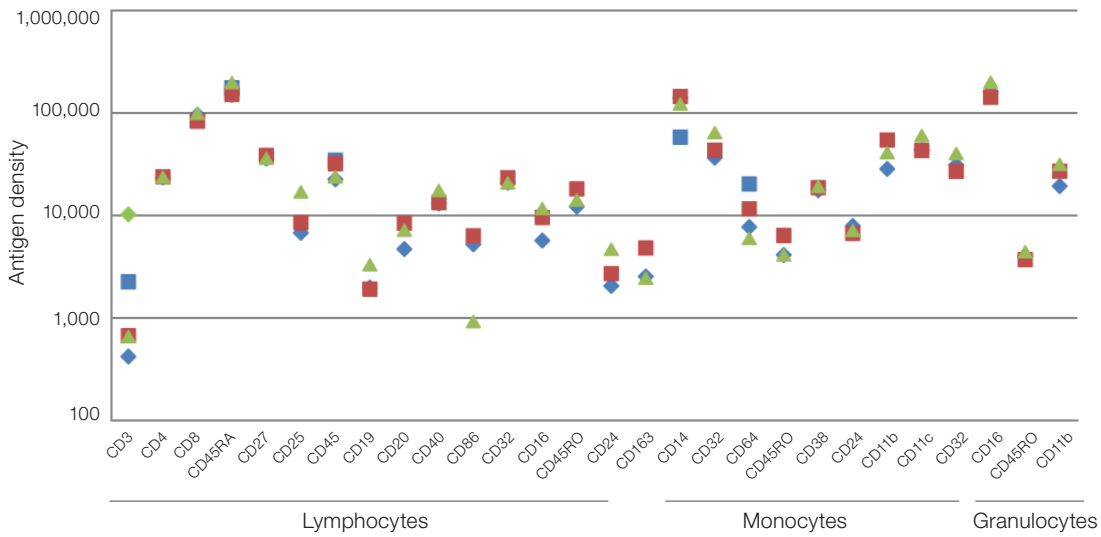
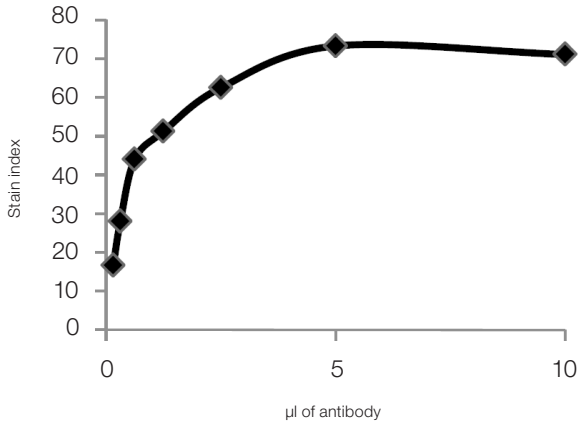


Fig. 4. Data from 21 common human markers. Geometric mean fluorescence intensity was obtained and converted into antigen density for each surface marker and plotted on a log scale. The results of up to four independent experiments on normal human blood donors are shown. Data acquired on the ZE5 Cell Analyzer and analyzed in FCS Express software.

Measure Antigen Density in Four Easy Steps

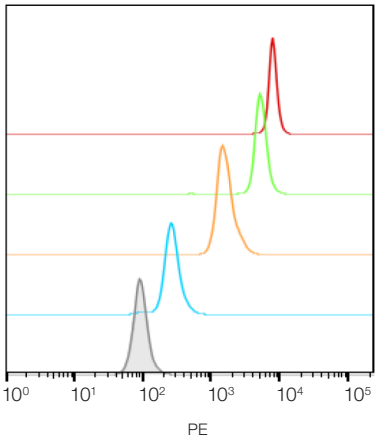
1 Titrate your antibody

Find the optimal stain index by titrating your antibody.



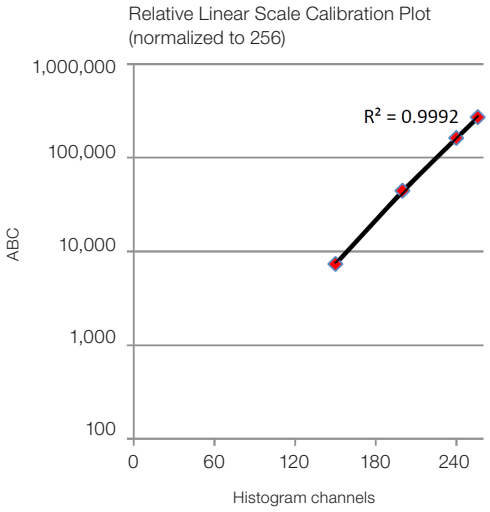
2 Stain your beads

Ensure your beads are saturated. Greater fluorescence can be seen in beads with increased antibody binding capacity (ABC).



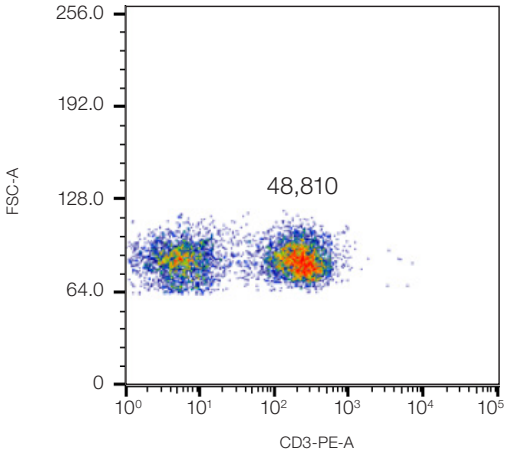
3 Create a standard curve

Use half height/full width gates to measure the geometric mean to create a standard curve of fluorescence against ABC.



4 Measure the antigen density

Using the geometric mean fluorescence intensity of the positive population, the antigen density can be calculated for any marker on your cell of interest.



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