CD25

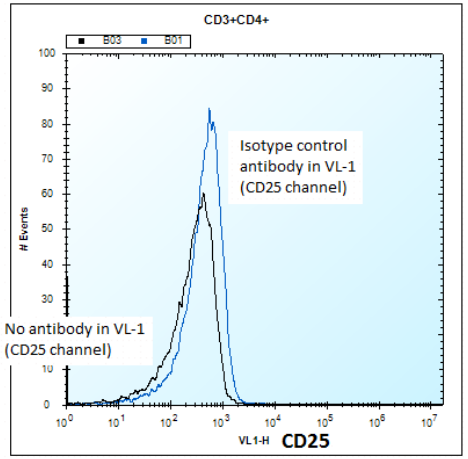
Human PBMCs gated for CD3+CD4 positive to define T helper cells.

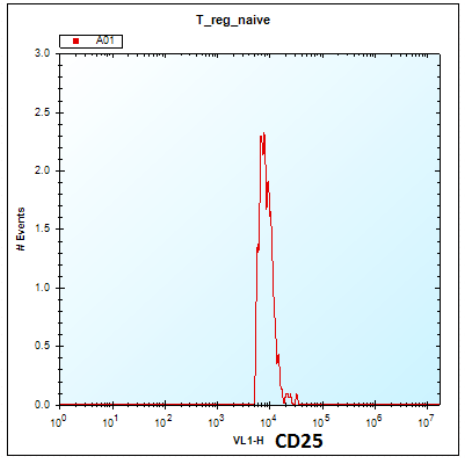
Within CD3+CD4+, T_regs and T_helper populations gated based on CD127 and CD25 (see plot, linear scale used to avoid losing cells where either marker was negative because of compensation).

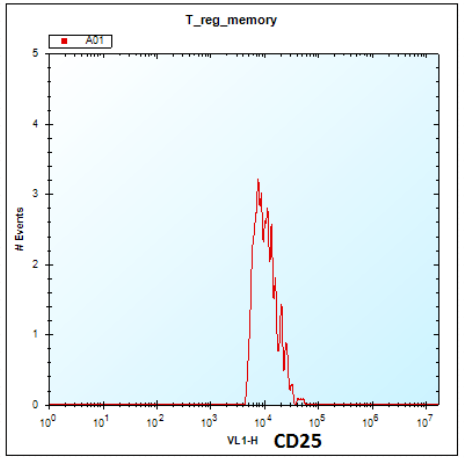
Since CD25-high is part of the definition of T_regs, it is not possible to define a matched population stained with isotype control antibody. Instead we use CD3+CD4+ cells stained with control antibody or stained with no antibody in that channel (top left plot).

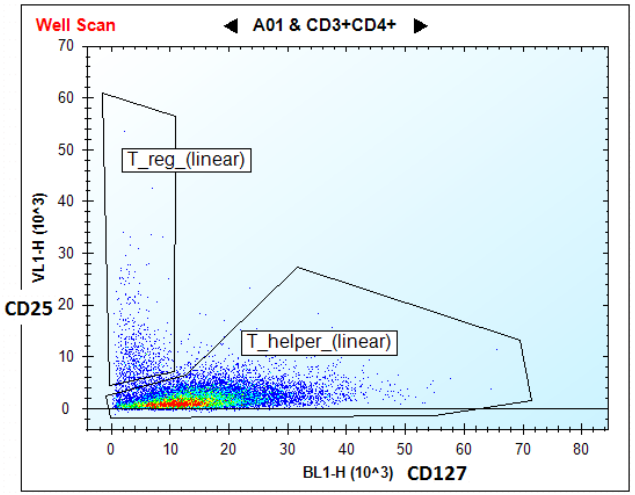
T_reg and T_helper were additionally gated into memory and naïve based on CD45RA expression.

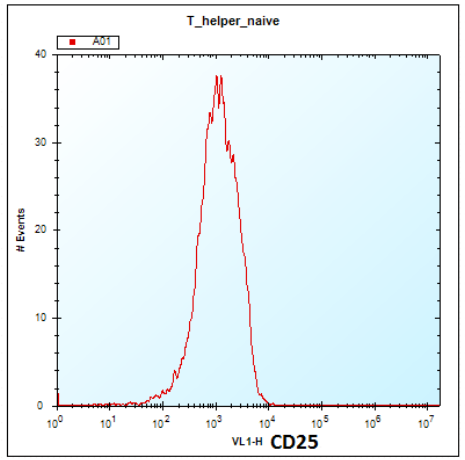
FCS files provided for CD3+CD4+ unstained, isotype control and stained for CD25 (in each case all other antibodies are unchanged). FCS files also provided for each subpopulation stained for CD25.

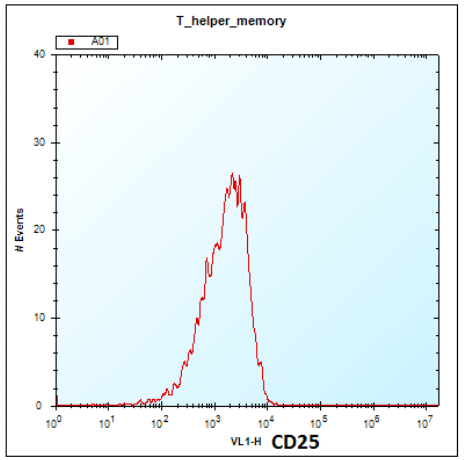












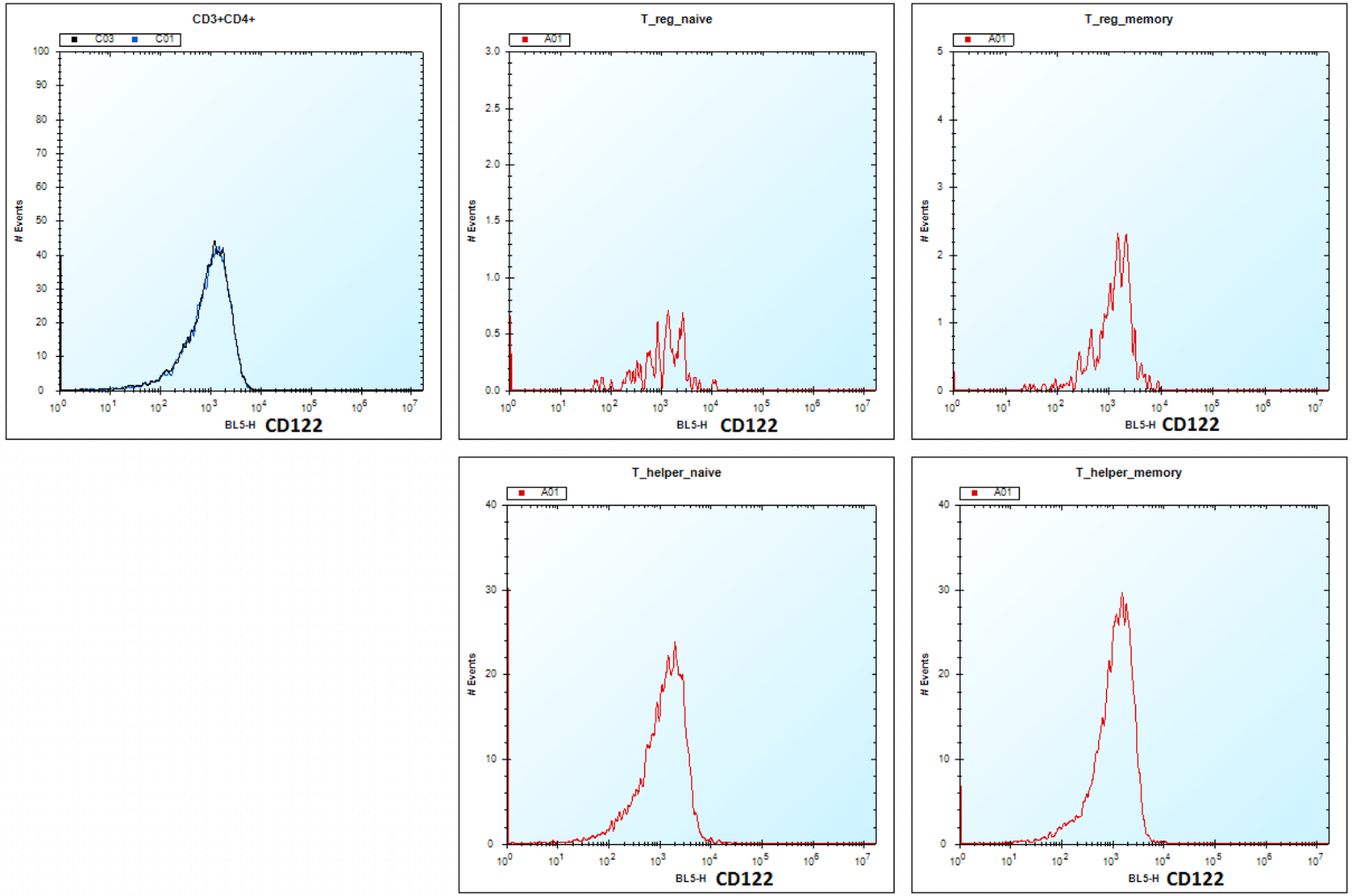
CD122

CD122 is challenging because the level is very low in these cells. Isotype control and CD122 stains are almost indistinguishable.

Log plots are not entirely representative because the distribution includes small negative values (negative values occur because of uncertainty in compensation measurements from abundant markers).

FCS files provided for CD3+CD4+ unstained, isotype control and stained for CD122 (in each case all other antibodies are unchanged). FCS files also provided for each subpopulation, unstained, isotype control, and stained for CD122.

Because CD122 is not used to gate populations, each population can be compared to a directly matched population in the control wells. The down-side is that rare populations are only represented by a few hundred counts, potentially increasing uncertainty. We are not sure whether it's better to define the negative control based on a large number of CD3+CD4+ cells, or else a small number from exactly matched population.



CD122

Cells stained for CD132, with unstained and isotype control stains used as controls.

FCS files provided for CD3+CD4+ unstained, isotype control and stained for CD132 (in each case all other antibodies are unchanged). FCS files also provided for each subpopulation, unstained, isotype control, and stained for CD132.

Because CD132 is not used to gate populations, each population can be compared to a directly matched population in the control wells. The down-side is that rare populations are only represented by a few hundred counts, potentially increasing uncertainty. We are not sure whether it's better to define the negative control based on a large number of CD3+CD4+ cells, or else a small number from exactly matched population.