

Abstract

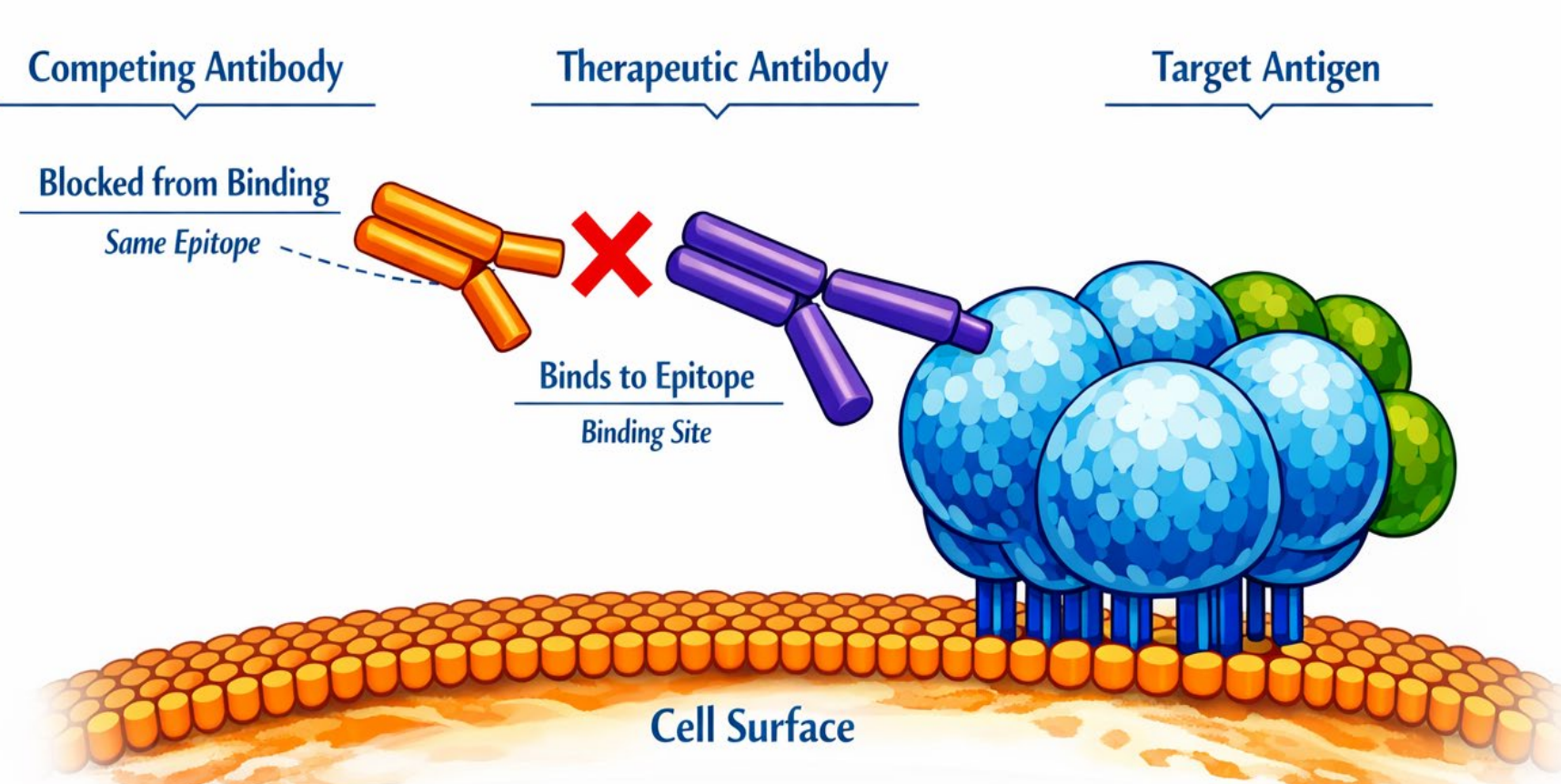
Blood samples containing therapeutic antibodies may pose a problem for researchers, as the therapeutic antibody may mask epitopes for the researcher's target. This interference is a major problem for identifying key cell populations and for analysis of target expression level. Identifying antibody clones that do not compete for binding with therapeutic antibodies is critical to overcome this problem. To this end, we have identified non-competing clones and validated them for flow cytometry in the presence of cognate therapeutic antibodies for major targets such as CD38, PD-1, CD22, HER-2, EGFR, and CD25. These flow cytometry reagents showed strong binding that was minimally affected by pre-treatment of cells with up to 40x concentration of the unconjugated therapeutic antibody. In addition, we present a list of clones that we offer for other targets that have been tested or reported in the literature as non-competing clones. Both competing and non-competing clones have utility in receptor-occupancy assays, so we also demonstrate clones that are blocked by cognate therapeutic antibodies. These data on competing and non-competing clones will allow researchers to carefully select the correct antibody for their specific purpose in studying these major biologics.

Background

Competition Between Therapeutic and Analytical Antibodies

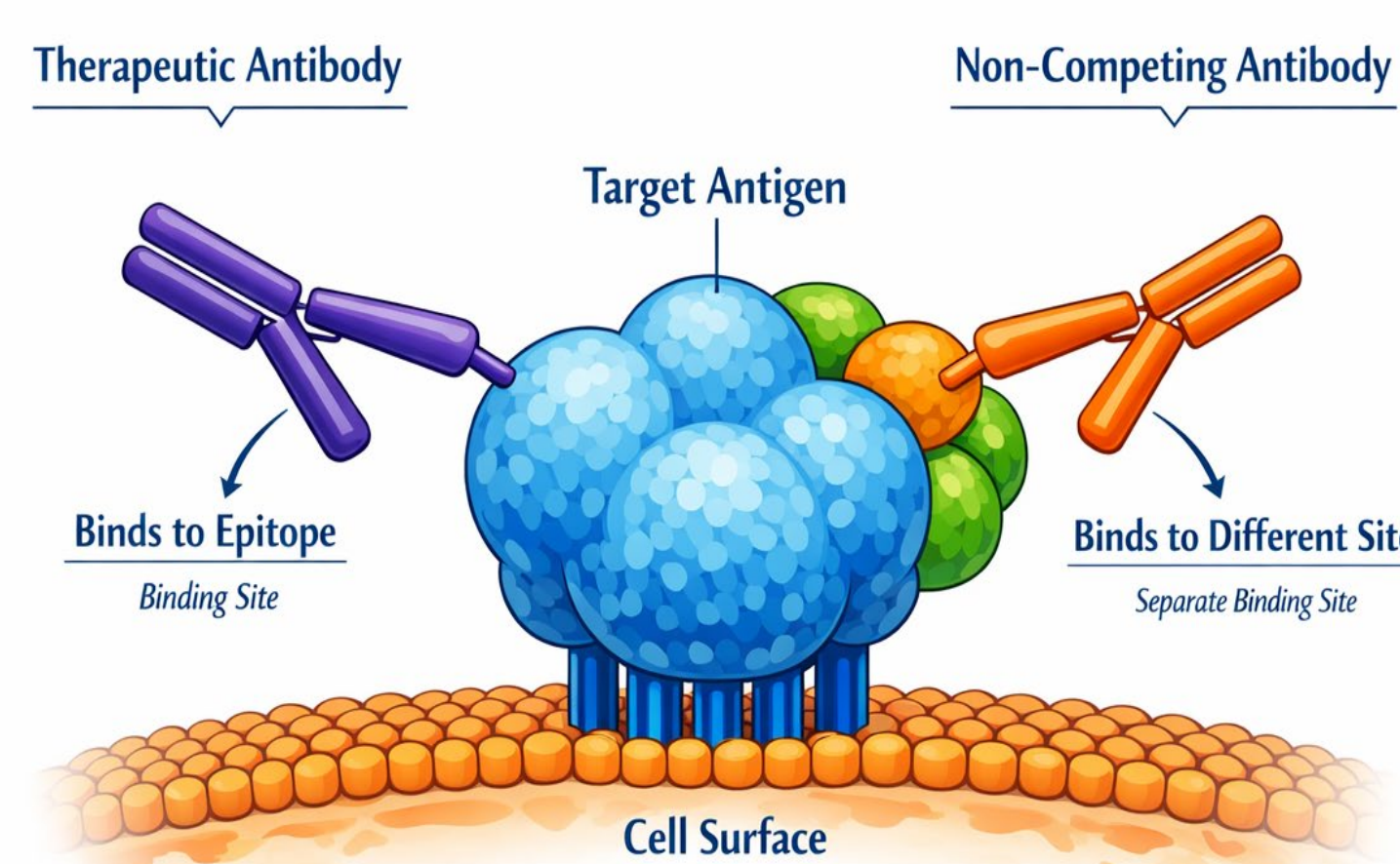
A) Competing Antibody Clone

- Epitope Analysis
- Receptor-occupancy Assays



B) Non-competing Antibody Clone

- Cell Population Identification
- Target Expression Level Assessment
- Target Up/down-regulation
- Receptor-occupancy Assays



Results

CD38 Non-competing Clone JK-2

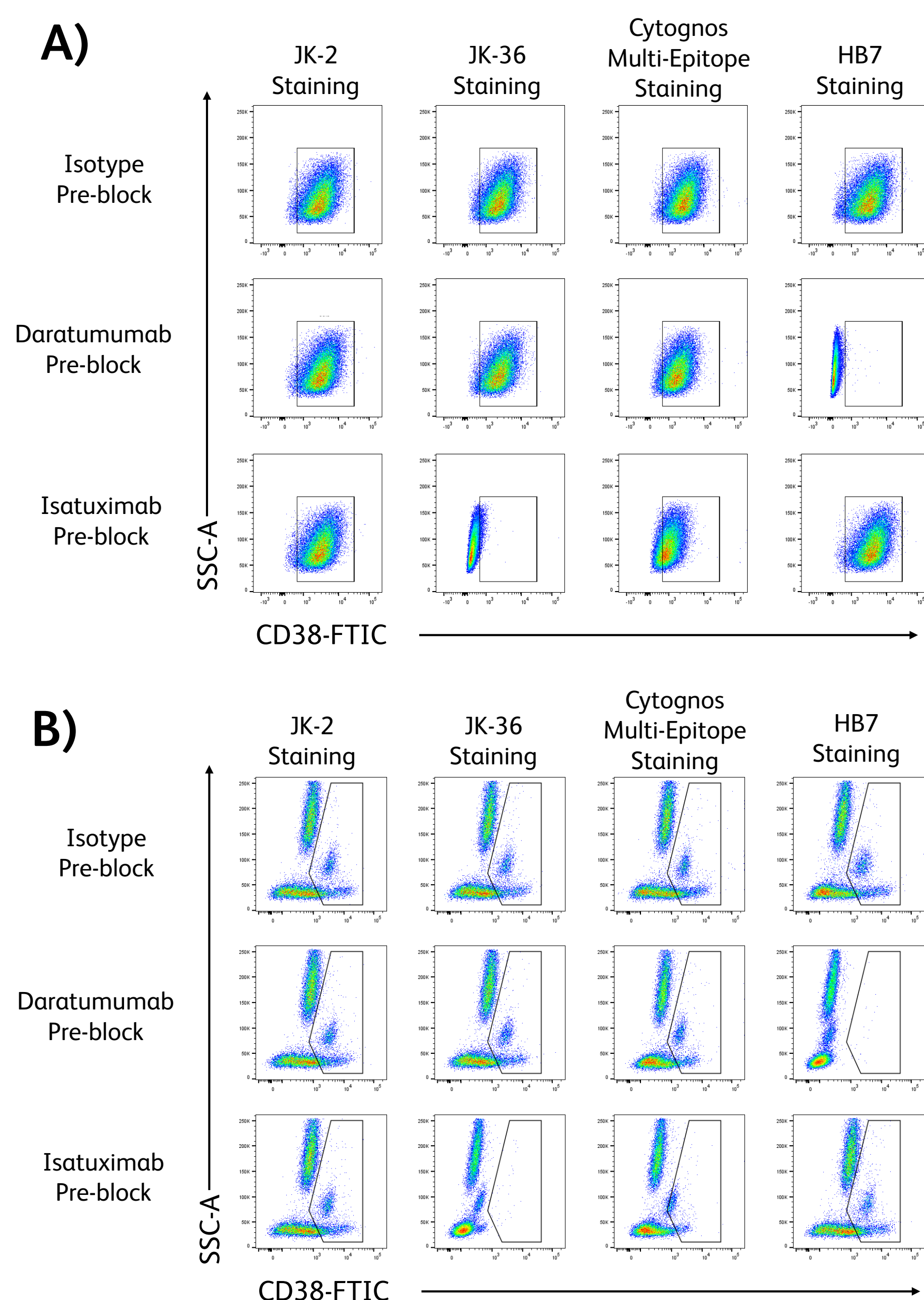


Figure 1. RPMI-8226 human myeloma cells (A) or lysed whole blood (LWB) from a healthy human donor (B) were pre-blocked with Daratumumab Biosimilar, Isatuximab Biosimilar, or Isotype Control antibody (40x relative to staining antibodies) for 30 minutes at RT prior to staining with indicated anti-CD38 dye-conjugated antibodies to identify non-competing clones. Similar results to LWB were obtained with healthy donor bone marrow.

PD-1 Non-competing Clone 135C12

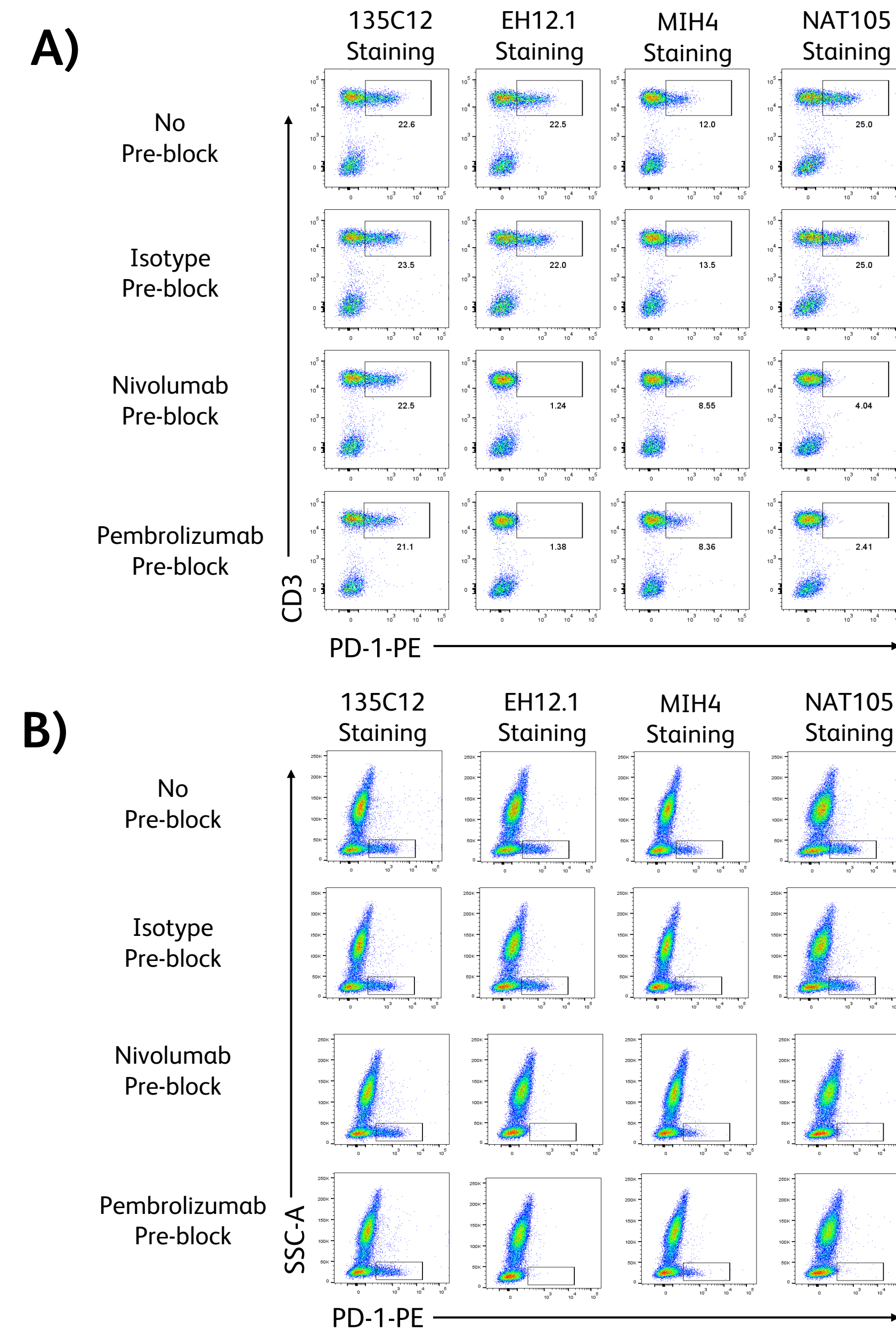


Figure 2. Lysed whole blood from a healthy donor was pre-blocked with Nivolumab Biosimilar, Pembrolizumab Biosimilar, or Isotype Control antibodies (40x relative to staining antibodies) for 30 minutes at RT prior to staining with anti-CD3 and indicated anti-PD-1 dye-conjugated antibody clones. (A) CD3 vs. PD-1 (B) SSC vs. PD-1

CD22 Non-competing Clone RFB4

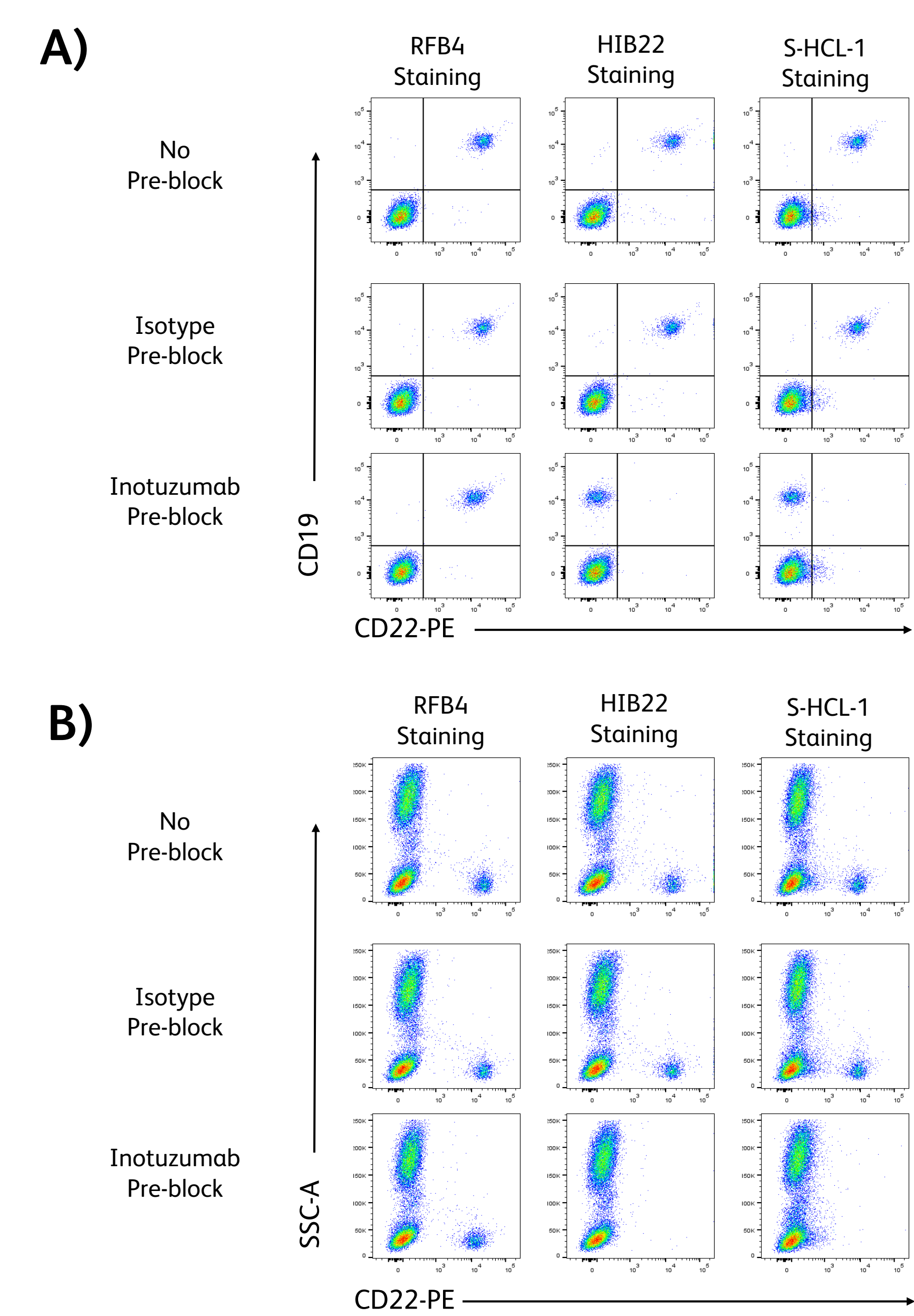
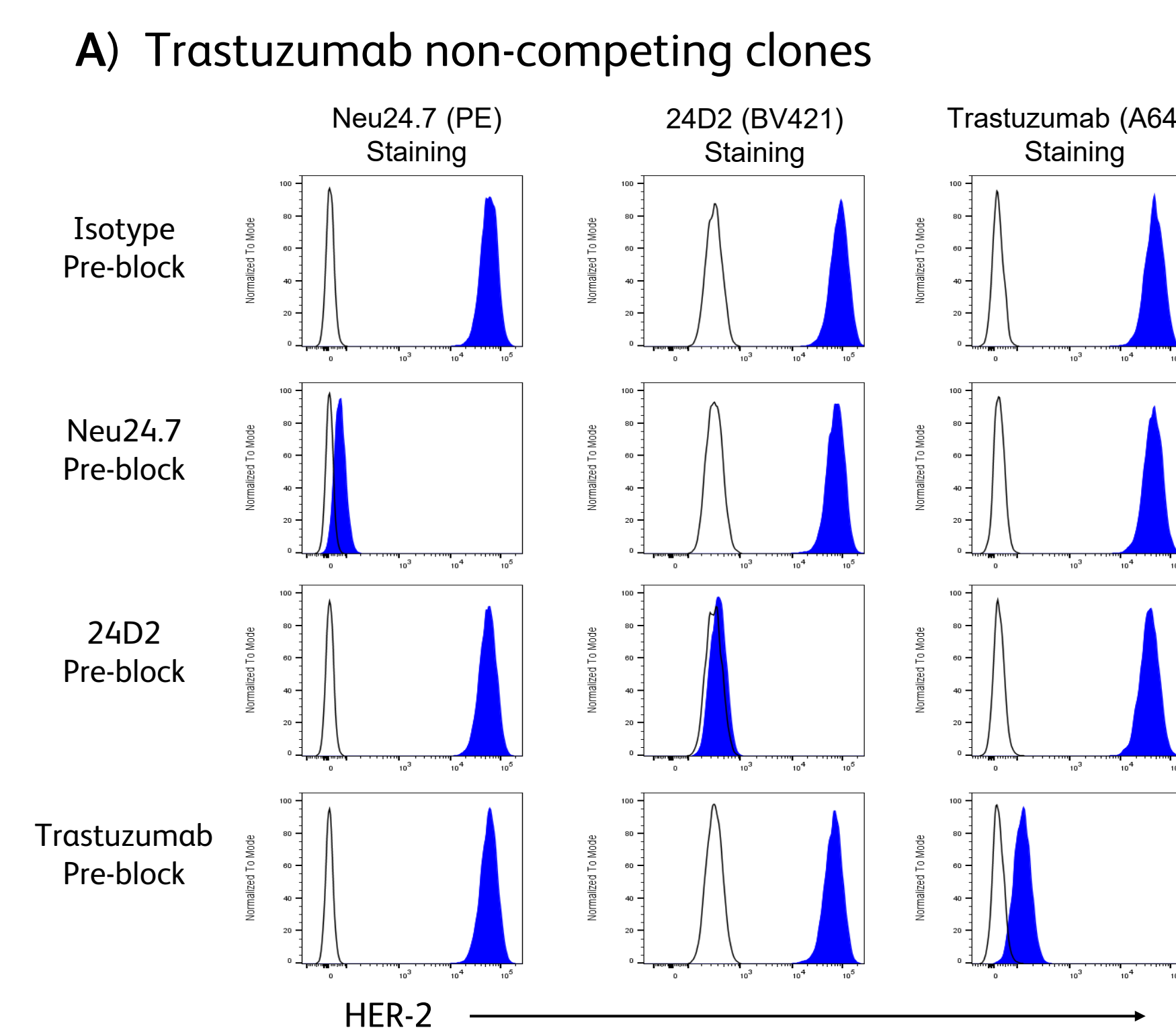


Figure 3. Lysed whole blood from a healthy donor was pre-blocked with Inotuzumab Biosimilar or Isotype Control antibody (40x relative to staining antibodies) for 30 minutes at RT prior to staining with anti-CD19 and indicated anti-CD22 dye-conjugated antibody clones. (A) CD19 vs. CD22 (B) SSC vs. CD22.

Her-2 Non-competing Clone 24D2



B) Trastuzumab-induced HER-2 downregulation

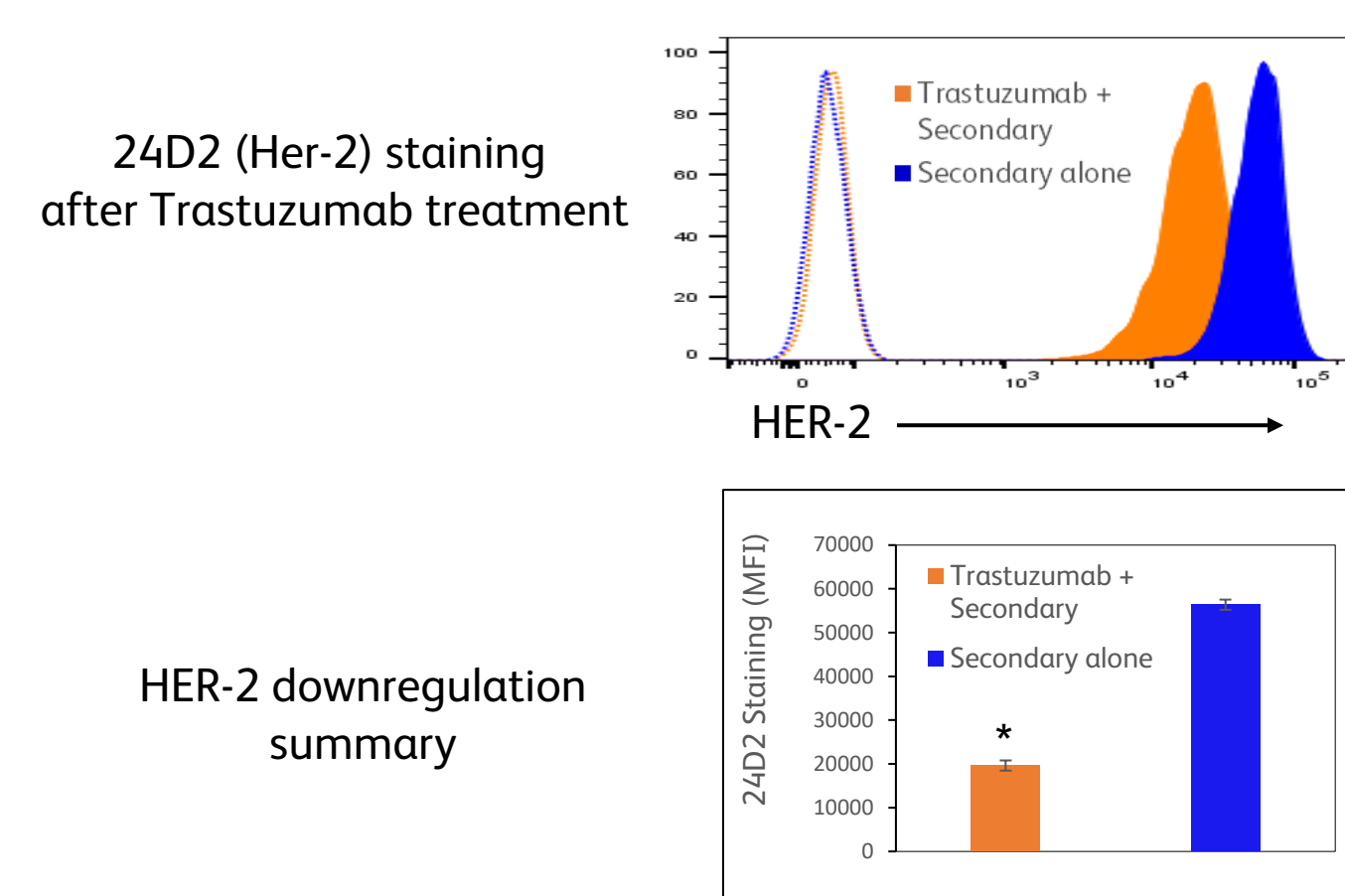


Figure 4. (A) SK-BR-3 cells were pre-blocked with NA/LE Trastuzumab Biosimilar (filled) or Isotype (open lines) to identify non-competing HER2 clones; neither 24D2 nor Neu24.7 clones appeared grossly affected by Trastuzumab Biosimilar binding. (B) Potential modulation of HER2 by Trastuzumab was investigated by treating with Biosimilar and secondary antibody for 44h, followed by staining for HER2 with a non-competing fluorochrome-conjugated clone. *p-value < 0.00005

EGFR Non-competing Clone EGFR.1

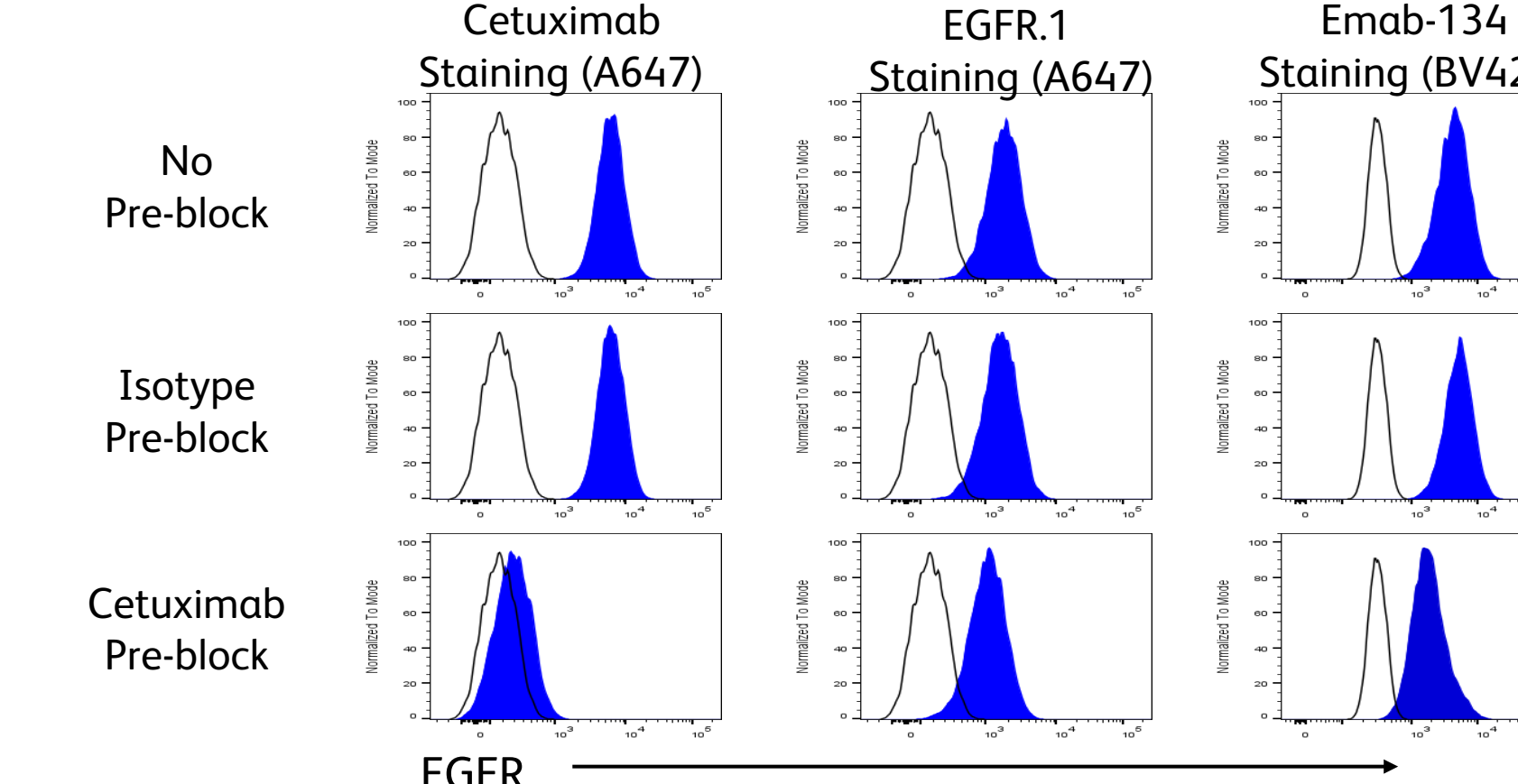


Figure 5. HEK293T cells were pre-blocked with 40x Cetuximab Biosimilar (filled) or Isotype (open lines) before staining with indicated anti-EGFR dye-conjugated antibodies to identify non-competing clones. Emab-134 shows partial competition.

CD25 Non-competing clone M-A251

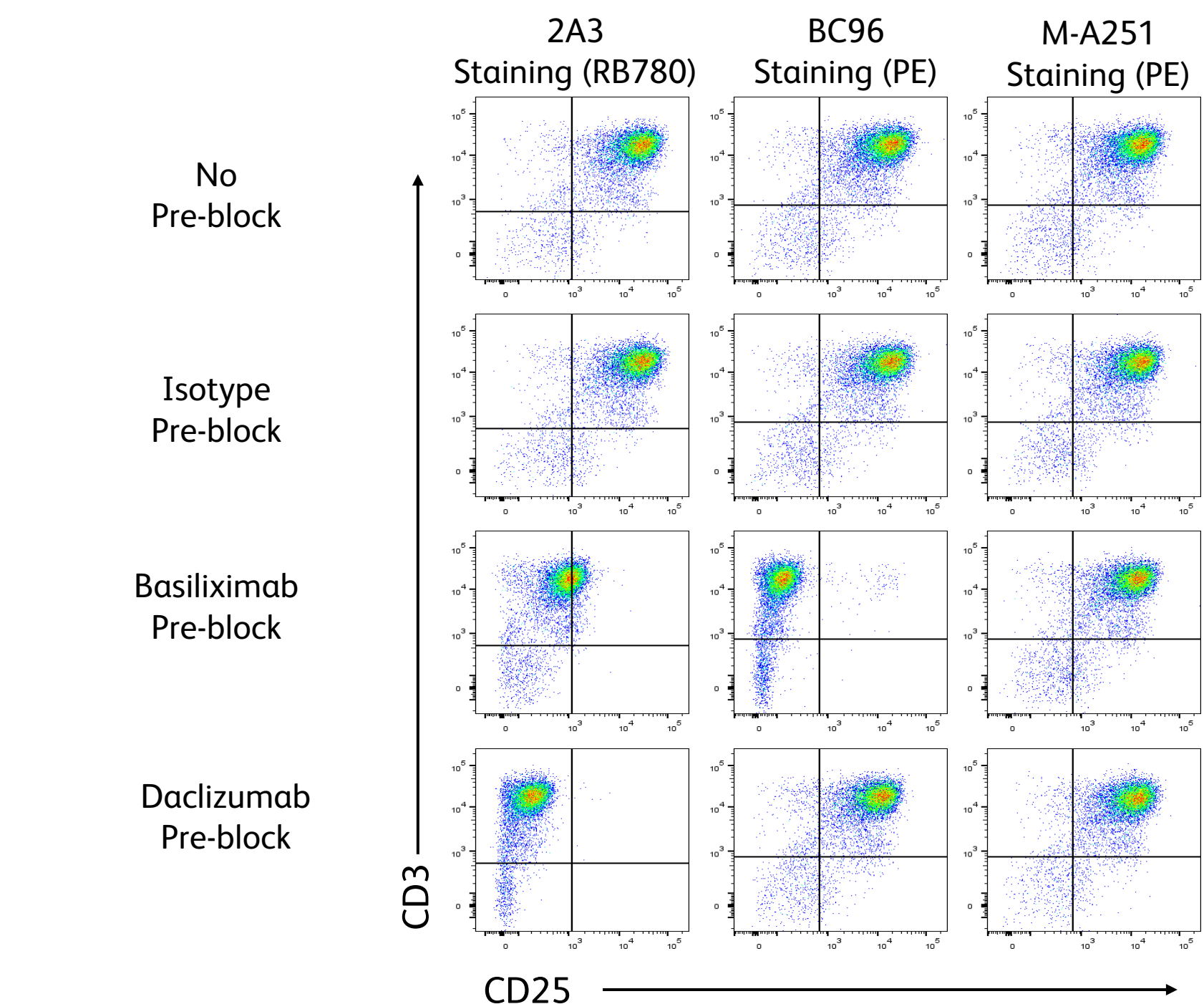


Figure 6. Human peripheral blood mononuclear cells (PBMC) were stimulated for 3 days with Phytohemagglutinin (PHA). Cells were then pre-blocked with Basiliximab Biosimilar, Daclizumab Biosimilar, or Isotype Control antibodies (40x relative to staining antibodies) prior to staining with anti-CD3 and indicated anti-CD25 dye-conjugated antibodies to identify non-competing CD25 clones.

Additional Non-competing Clones

Tested at BD

Target	Therapeutic	BD Non-competing Clones	BD Competing Clones
CD33	Gemtuzumab	HIM3-4*	P67.6 WM53
CD49d ($\alpha 4$ Integrin)	Natalizumab	9F10	PS/2 L25 Natalizumab297.rMAb Biosimilar
$\alpha 4\beta 7$ Integrin	Vedolizumab	L25 PS/2 9F10 FIB905	Vedolizumab297.rMAb Biosimilar
CD125	Benralizumab	A14	
CTLA-4	Ipilimumab	BNI3	
VEGFR2	Ramucirumab	BB13-1044	
CD126	Tocilizumab	M5	

* Minimal competition observed

Reported in Literature

Target	Therapeutic	BD Non-competing Clones	BD Competing Clones
CD3	Orthoclone OKT3	SK7	OKT3
PD-L1	Atezolizumab	29E.2A3	