

# Bright and Stable CD19 CAR Detection Reagent for 1-step Staining and Flow Cytometry

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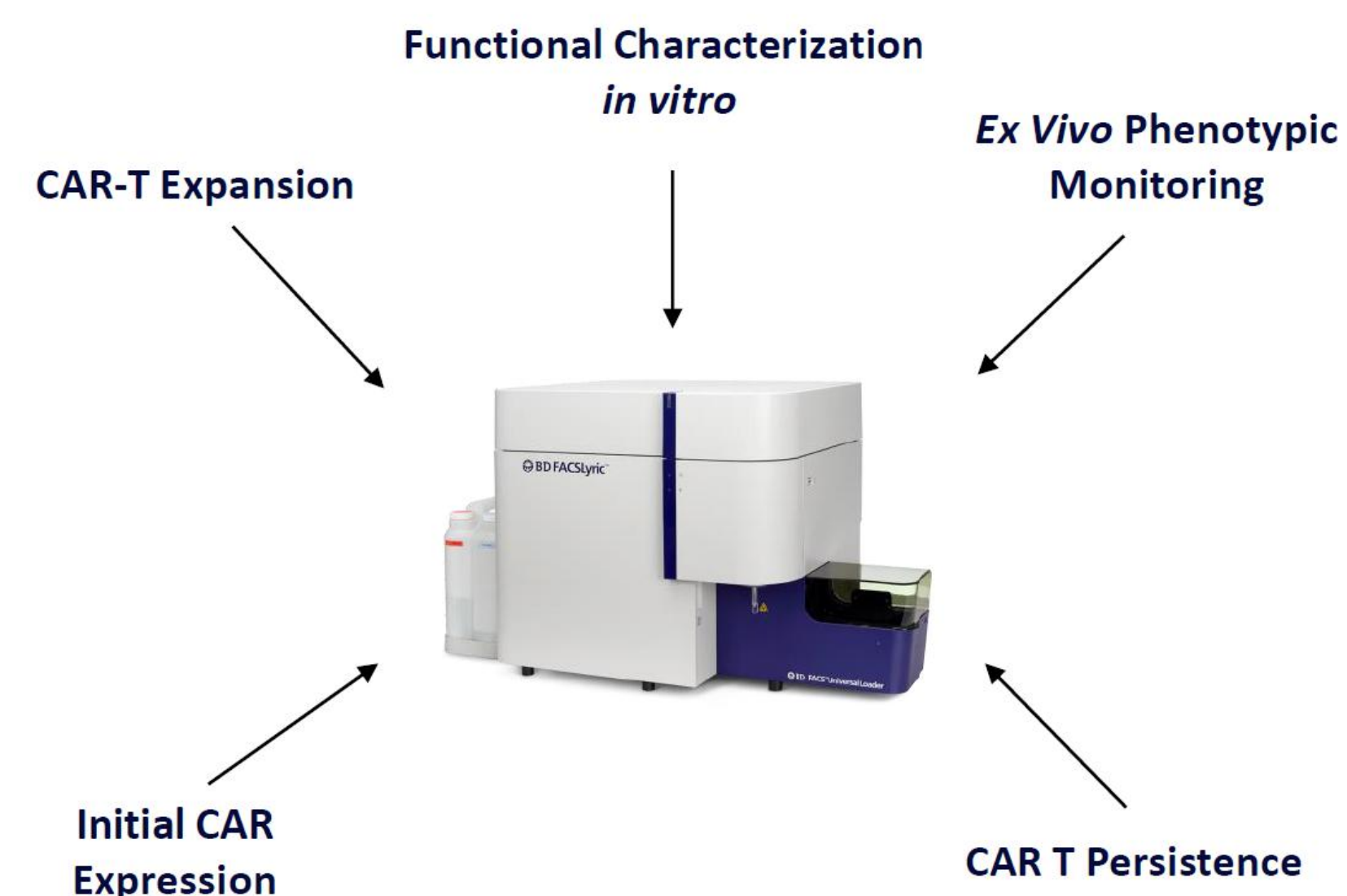
BD Biosciences

## Abstract

Flow cytometric identification and analysis of CD19 CAR-expressing cells is important throughout CAR discovery and translational workflows. However, surface CD19 CAR protein expression can vary widely, making accurate identification and analysis of CAR+ cells difficult. Furthermore, CD19 CAR detection using recombinant protein is difficult, since soluble CD19 protein is unstable. To overcome these challenges, BD<sup>®</sup> has developed an innovative bright and stable 1-step CD19 CAR detection reagent that is compatible with multiple fluorochrome options to accommodate varying panel needs and instrument constraints. Here, we show that the BD<sup>®</sup> CD19 CAR Detection Reagent specifically stains CAR-expressing CHO, Jurkat CAR-T, and primary human CD19 CAR-T cells. In addition to *in vitro* cell culture settings, our CD19 CAR Detection Reagent performs well on lysed whole blood, in multi-color panels, and shows compatibility with intracellular staining workflows. CAR-T cells added to donor PBMC were detected by this reagent at <0.1% proportions, showing potential value in research on CAR-T persistence. Importantly, background staining was minimal on CAR-negative blood cell populations, and CAR<sup>lo</sup> cells could be resolved from negative cells. Overall, our data indicate that this new reagent addresses the challenges of CD19 CAR+ cell detection and will empower CAR cell research.

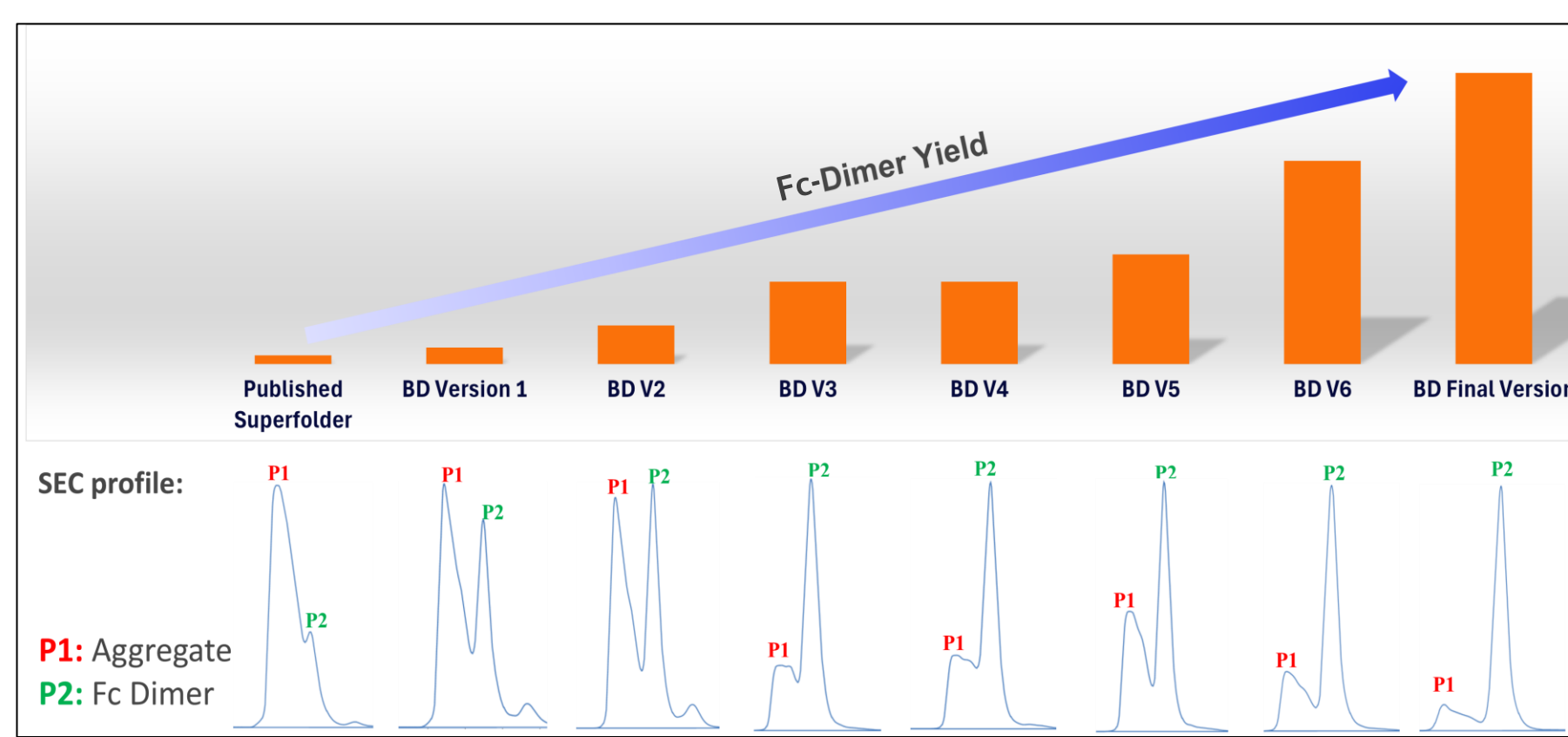
## Objective

### High-performance CD19 CAR Detection Reagent to Meet CAR T Workflow Needs

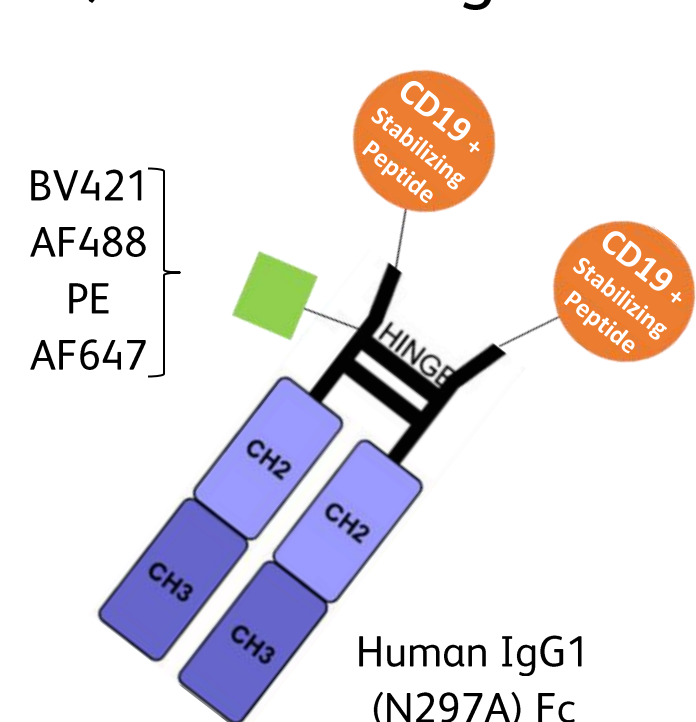


### Development of a Stable CD19 Reagent

#### A) Iterative improvement



#### B) Final Design

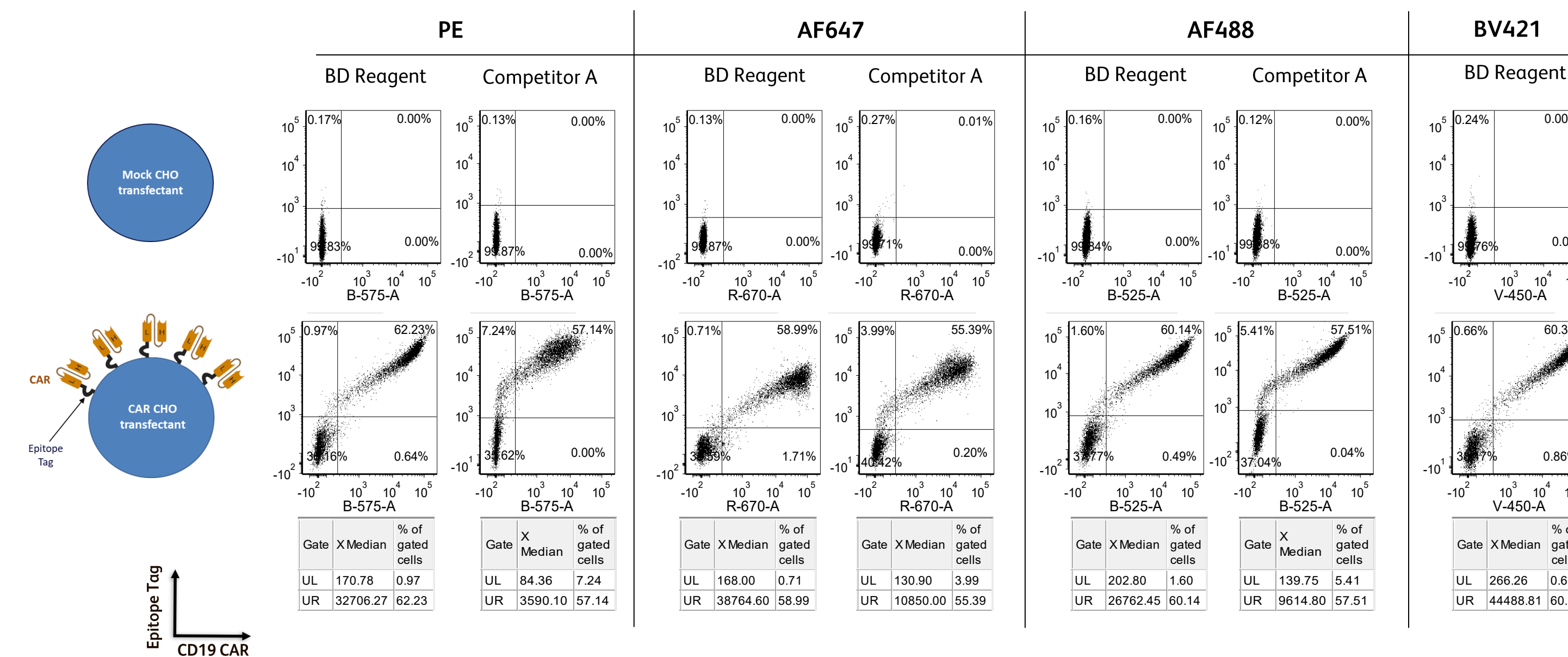


**Figure 1.** Development of a stable soluble CD19 CAR Detection Reagent. (A) Successive design iterations improved dimer yield >50-fold and dramatically reduced aggregation. (B) Final design of BD CD19 CAR Detection Reagent is a proprietary fusion protein containing CD19<sub>21-278</sub>, a stabilizing peptide, and huIgG1(N297A) Fc. These fusion protein dye conjugates have a shelf life >18 months at 4°C.

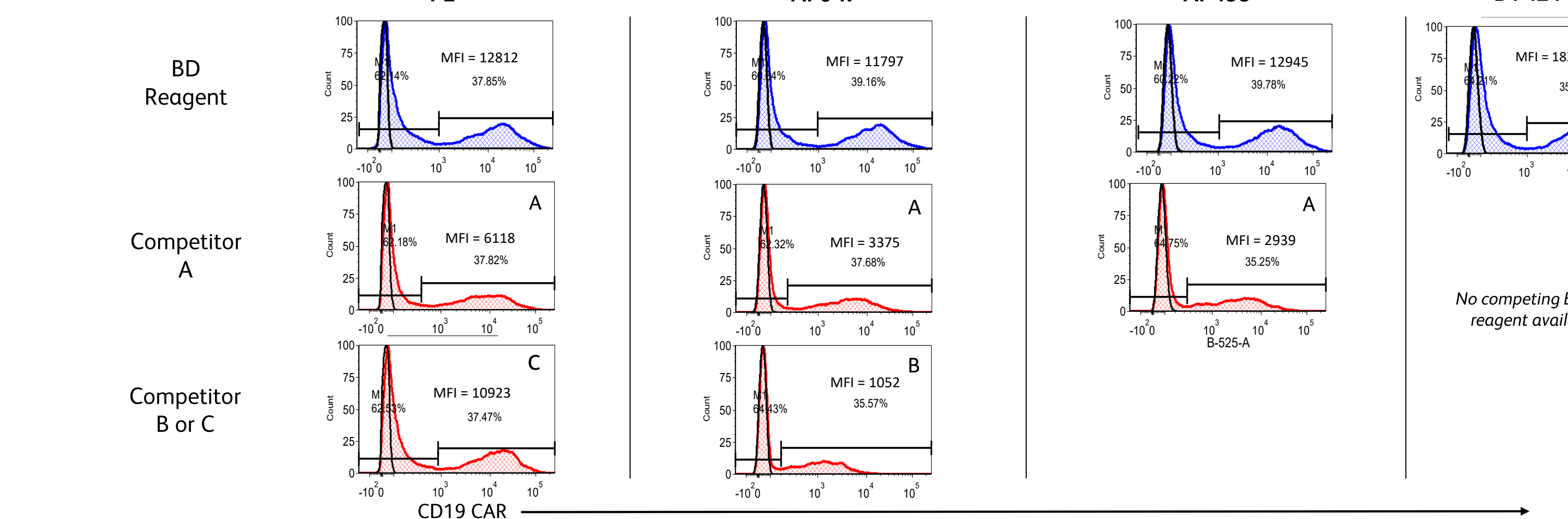
## Results

### Staining Performance

#### A) CHO-CAR Transfectants



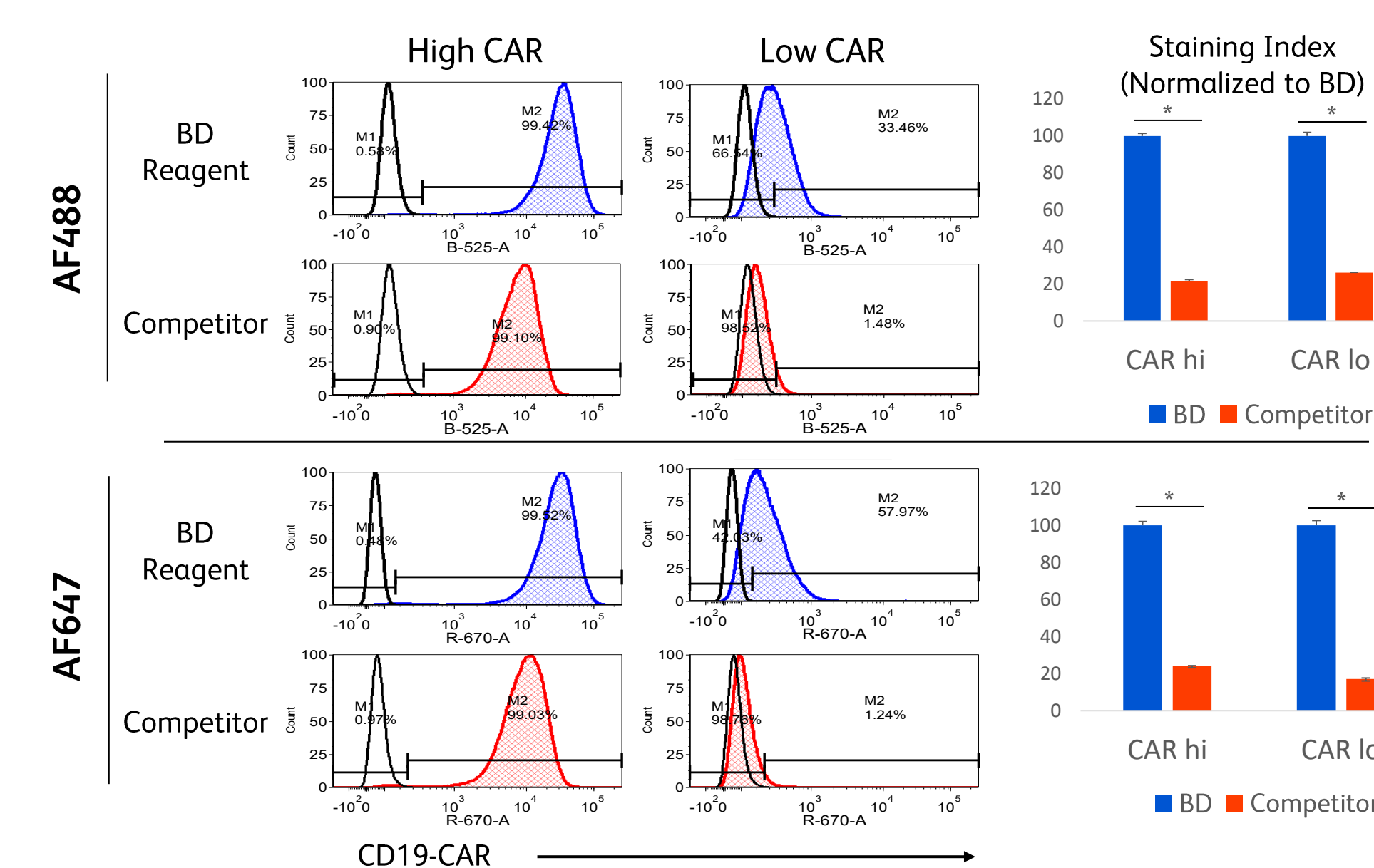
#### B) Primary CAR T



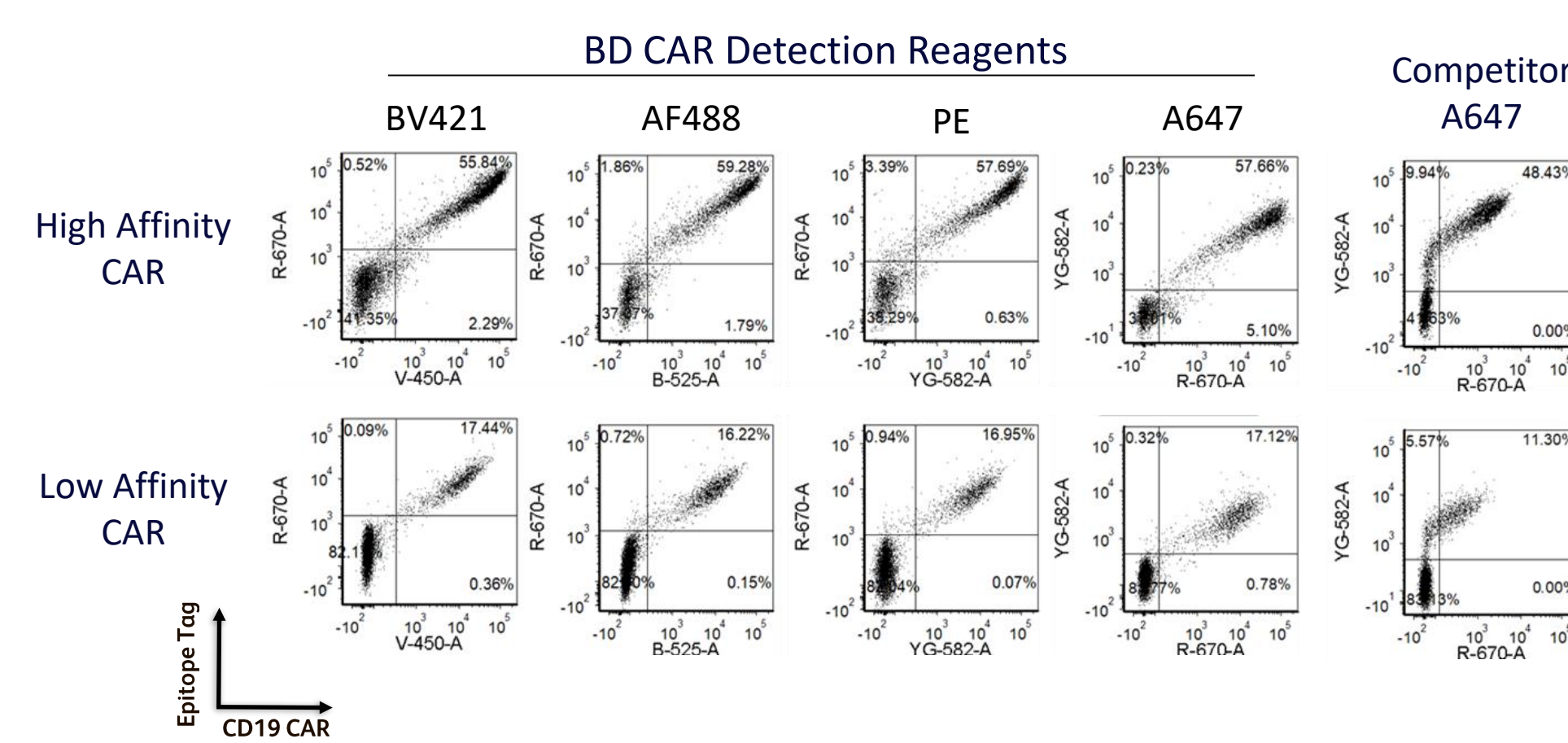
**Figure 2.** Staining Performance. (A) CHO cells were transfected with mock- or CD19-CAR and stained with BD or competitor CD19 CAR Detection Reagents (at recommended concentrations) with epitope tag antibody co-stain for 30 min at RT. (B) T cells purified by negative depletion from a healthy donor in the Associate Sample Collection Program (ASCP) were activated with anti-CD3/28 beads + IL-2 for 2d before infection with CD19-CAR lentivirus, followed by expansion for 7-10 days with IL-2 and cryopreservation. Thawed cells were stained with the indicated CAR T detection reagents at recommended concentrations.

### Sensitivity on low-level or low-affinity CARs

#### A) Staining on CAR<sup>hi</sup> and CAR<sup>lo</sup> Jurkat clones



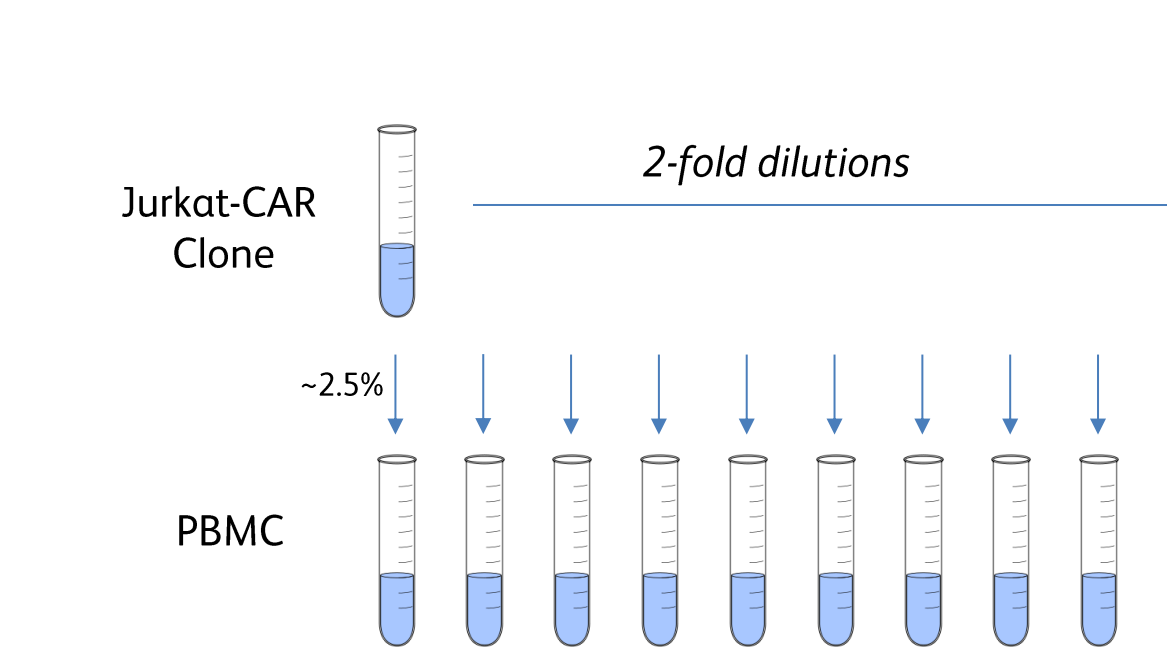
#### B) Staining on low-affinity CAR



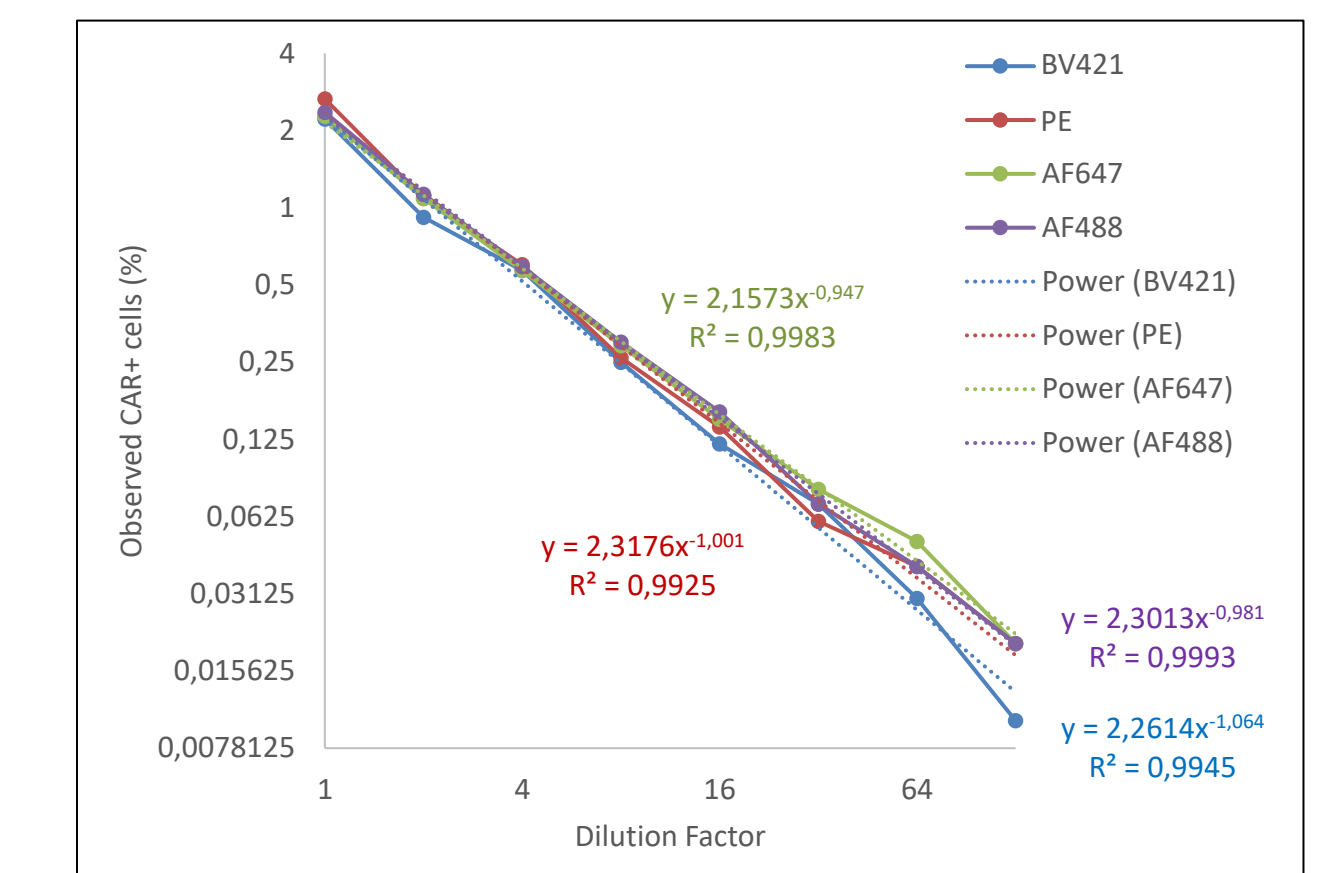
**Figure 3.** Detection of low CAR expression with CD19 CAR Detection Reagents. (A) Jurkat cells were infected with CD19 CAR lentivirus and single-cell cloned to obtain CAR hi and CAR lo clones. These were stained with BD vs. indicated competitor reagents at recommended concentrations for 30 min at RT. Histograms are representative data; bar graphs summarize data as % of BD staining index. Error bars are S.E.M. of triplicate staining wells. \*p-value < 0.00001 (B) CHO cells were transfected with high affinity (FMC63 scFv) or low affinity (CAT13.1E10 scFv) CD19-CAR and stained with BD or competitor reagents (at recommended concentrations) for 30 min at RT.

### Rare Cell Detection

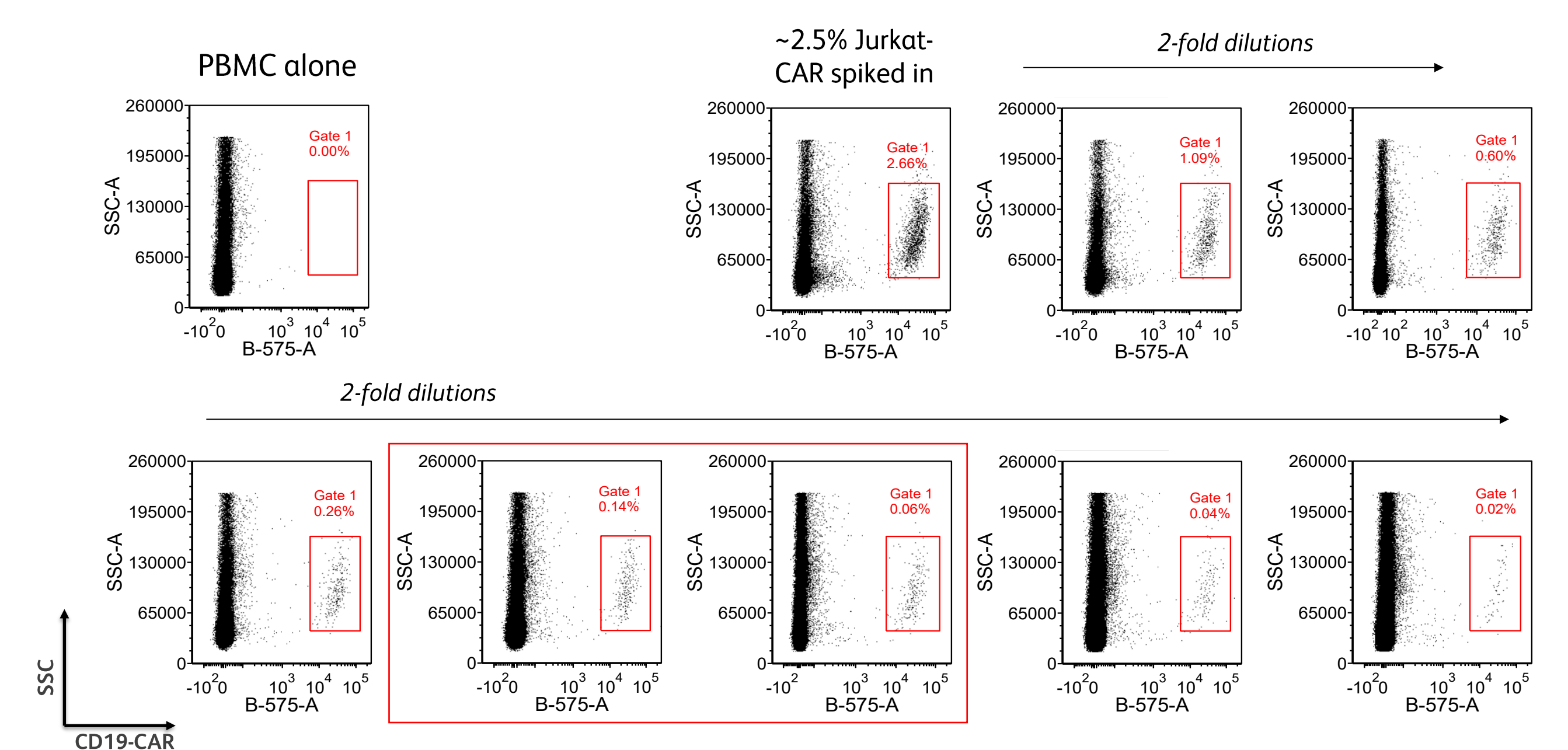
#### A) Model



#### C) Summary



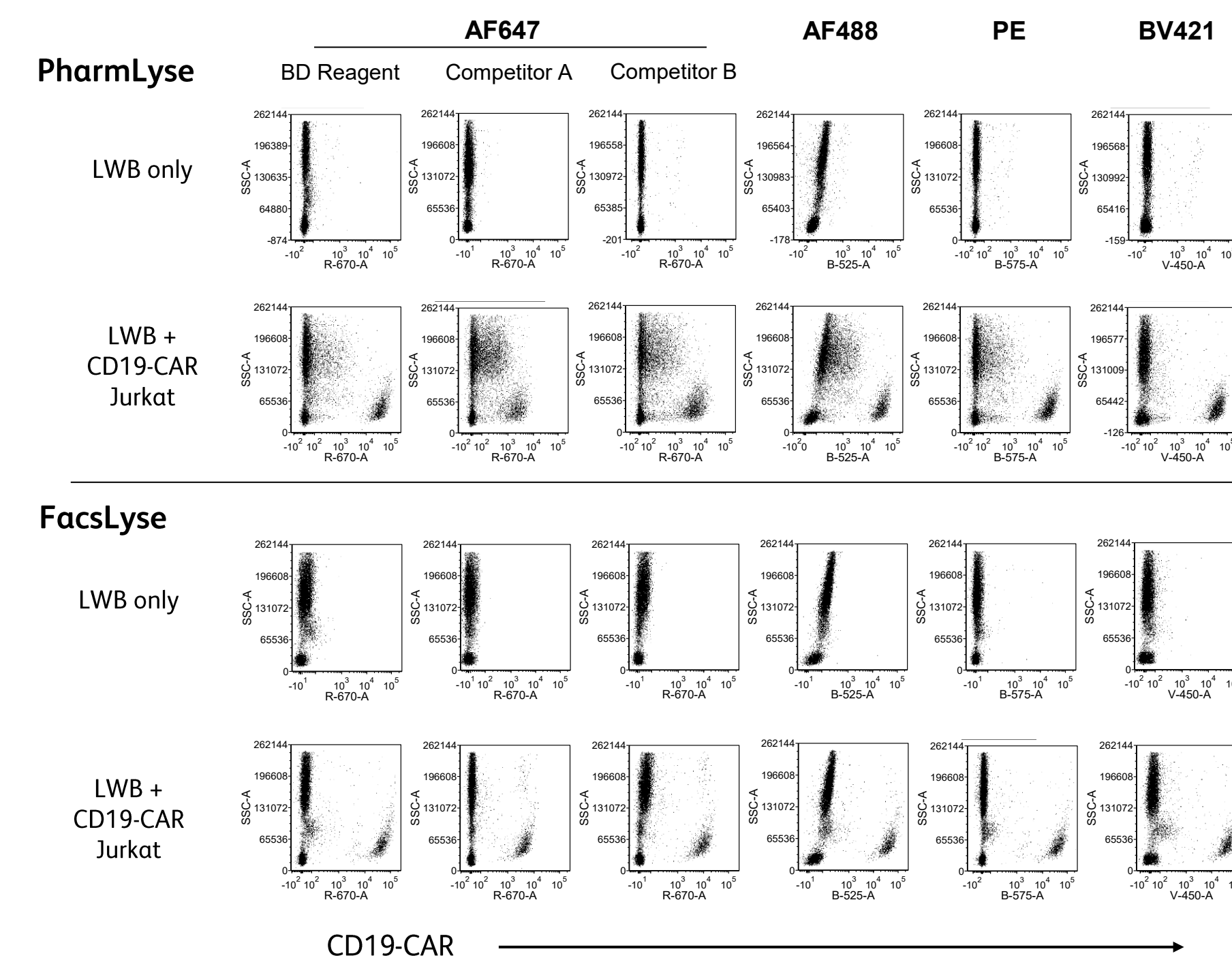
#### B) Representative Data (PE)



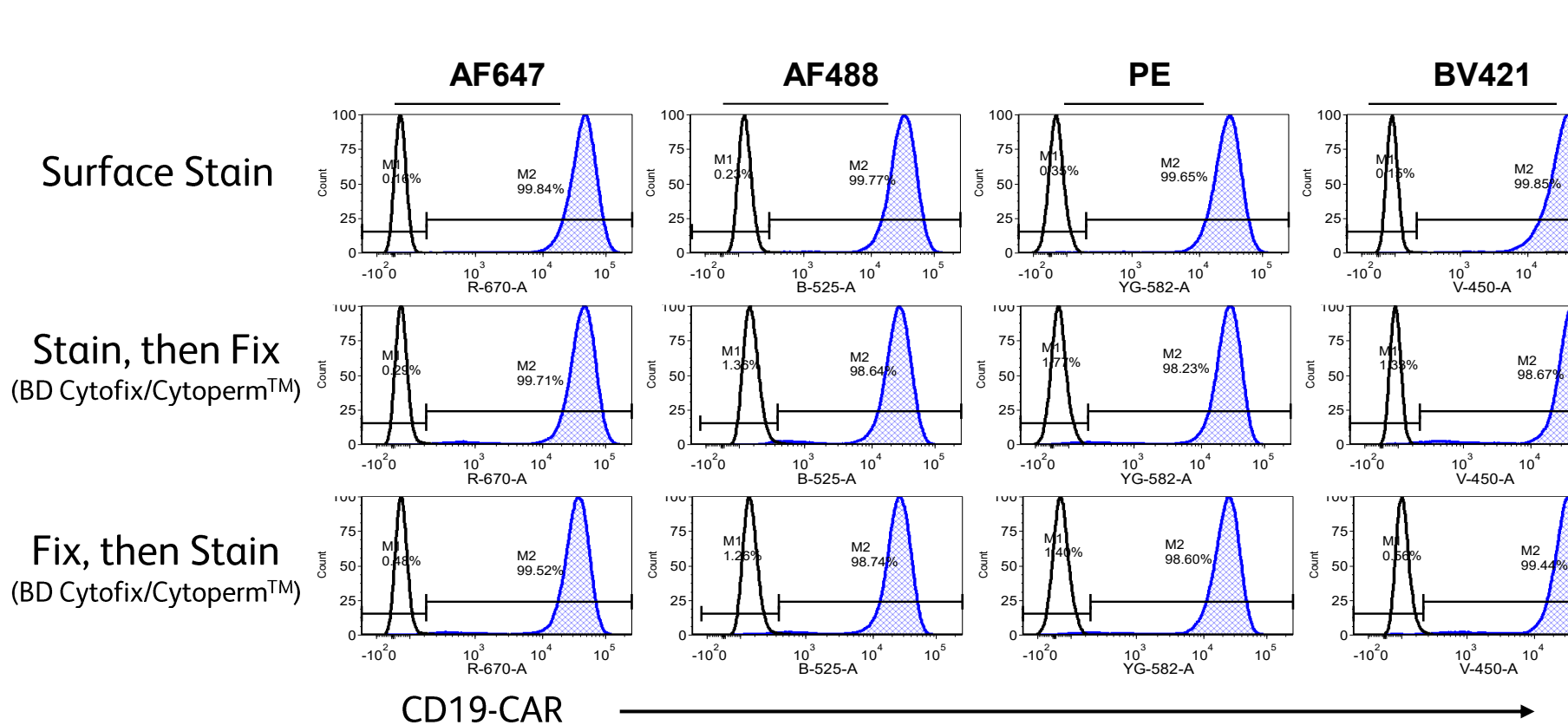
**Figure 2.** Rare Cell Detection. (A) Model. Jurkat-CAR clone isolated from CD19 CAR lentivirus-infected cells was spiked into PBMC from a healthy donor in the Associate Sample Collection Program (ASCP) at approximately 2.5% or one of 9 descending 2-fold Jurkat clone dilutions. (B) Representative Data (PE). Jurkat-CAR-spiked PBMC were stained with PE-conjugated BD CD19 CAR Detection Reagent at test size for 30 min at RT, following by flow cytometric analysis. Red box highlights detection of CAR+ cells at ≤0.1% of PBMC. (C) Summary. Observed percentages of CAR+ cells were plotted vs. predicted % based on 2-fold division of initial % to estimate ability to accurately detect CAR+ rare cells at ≤0.1%.

### Buffer Compatibility

#### A) Lysis Buffers



#### B) Fixation Buffers



**Figure 4.** Buffer Compatibility. (A) Lysed whole blood (LWB) from a healthy donor was spiked with CD19 CAR hi Jurkat clone from Fig. 3, and stained with BD or competitor CD19 CAR Detection Reagents at recommended concentrations, followed by lysis with BD Pharm Lyse™ or BD FACSTM Lysing Solution. (B) CD19 CAR hi clone from Fig. 3 was stained using BD CD19 CAR Detection Reagents and BD Cytofix/Cytoperm™ Permeabilization Solution using the indicated protocol.

### CD19 CAR Reagent Summary

- Advantages over linker Abs
  - Antigen binds CAR consistently
  - Specificity allows dual/tandem CAR study
- 4 direct conjugates for flexibility
- Very competitive performance
- Low background
- Excellent rare cell detection
- Sensitivity to detect low level/affinity CAR
- Long shelf-life (at least 2 yrs at 4°C)
- Broad Compatibility
  - Lysis buffers
  - Permeabilization buffer
  - Pre-fixed cells

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