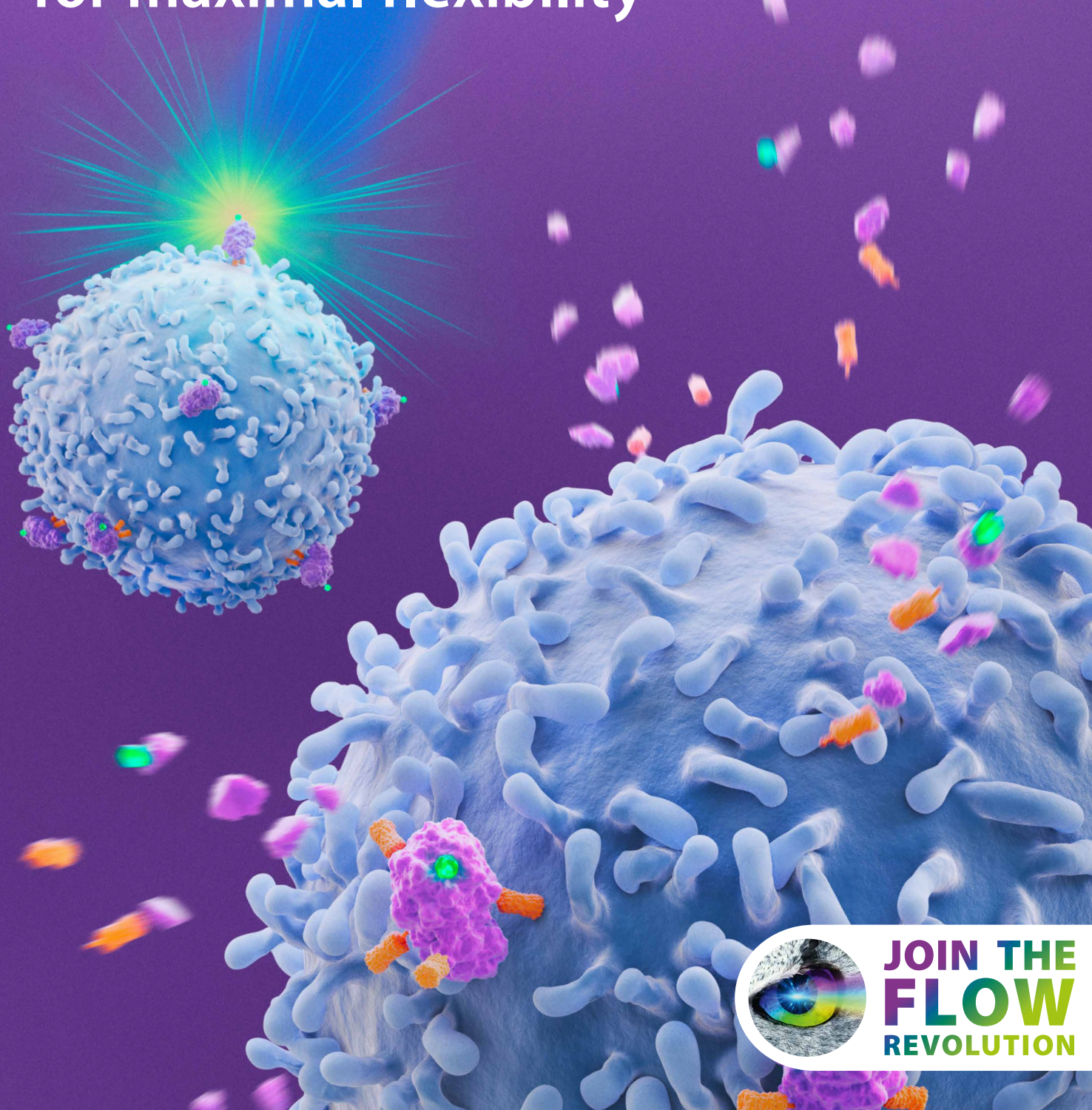


REAl ease[®] Fluorochrome Technology

Label-free cells after cell sorting for maximal flexibility



**JOIN THE
FLOW
REVOLUTION**

REAl ease[®] Releasable Antibodies for cell sorting

- The next step in flexibility for cell sorting
- Label-free cells for downstream applications
- Recombinantly engineered for reproducible results

The basic principle

REAl ease[®] Fluorochrome Technology (fig. 1) relies on recombinantly engineered antibody fragments that are, unlike conventional antibodies, characterized by low epitope binding affinities. A novel covalent conjugation approach allows for multimerization and fluorescent labeling of these antibody fragments. They form the so called REAl ease Fluorochrome Complex, which facilitates high-avidity cell binding that is comparable to conventional antibody fluorochrome conjugates (fig. 2). This novel and innovative conjugation chemistry allows for fast removal of the REAl ease Fluorochrome Complex from the cell surface by adding the REAl ease Release Reagent after flow sorting. This leads to disruption of the complex and thereby results in monomerization of the antibody fragments. Due to their engineered low binding affinity, they spontaneously dissociate from the cell surface. As a result, label-free cells can be obtained after cell sorting. The advantages?

1. Previously utilized fluorescence detection channels become available again, allowing for renewed targeting of additional epitopes for subsequent flow sorting cycles or downstream analyses.
2. Previously targeted epitopes are no longer blocked, enabling downstream analyses and applications that require free epitopes.

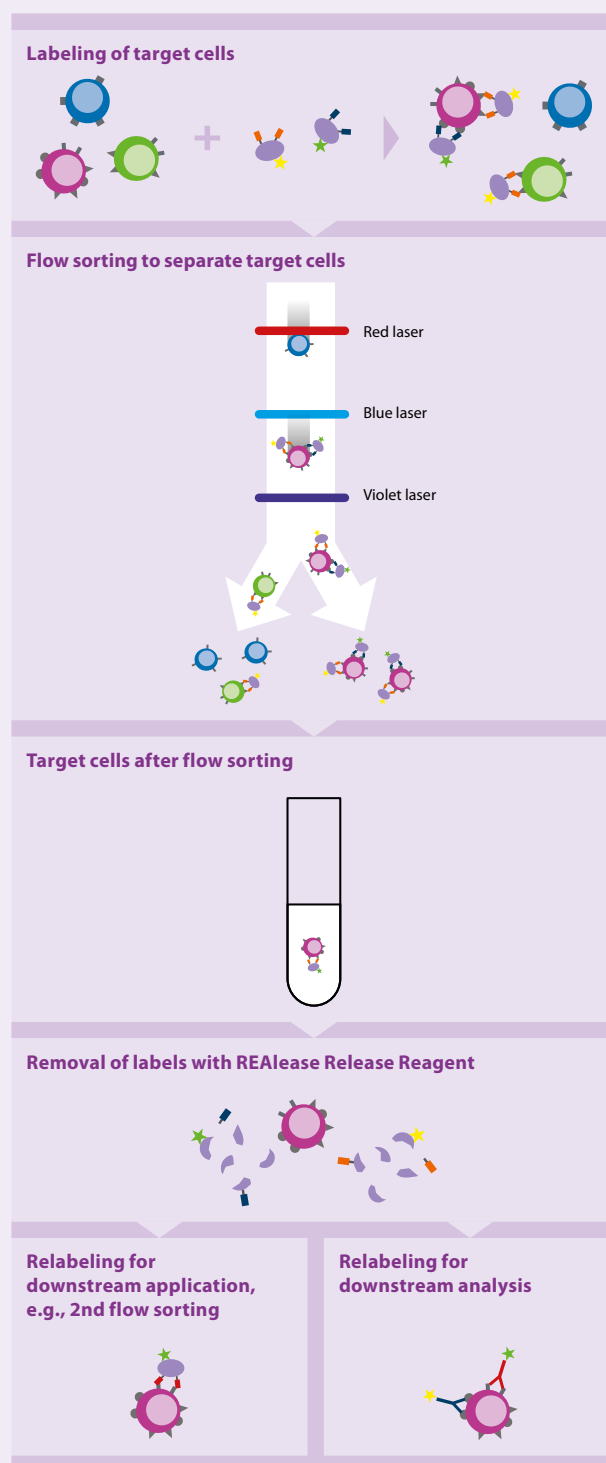
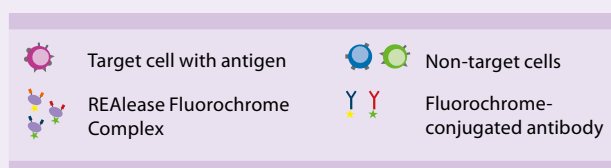


Figure 1: The principle of releasable antibody fluorochrome conjugates.

Uncompromised fluorescent antibody staining

The staining performance of REAlease® Antibodies is equivalent to conventional antibodies with comparable brightness and low non-specific binding (fig. 2A and B).

Efficient label release from target cells

The removal of REAlease Antibodies is highly efficient and selective with no detectable fluorescence signal remaining after the release step. Additionally, the labeling efficiency in the relabeling step is equivalent to the initial staining, demonstrating that epitopes become completely available again (fig. 2A, C and D).

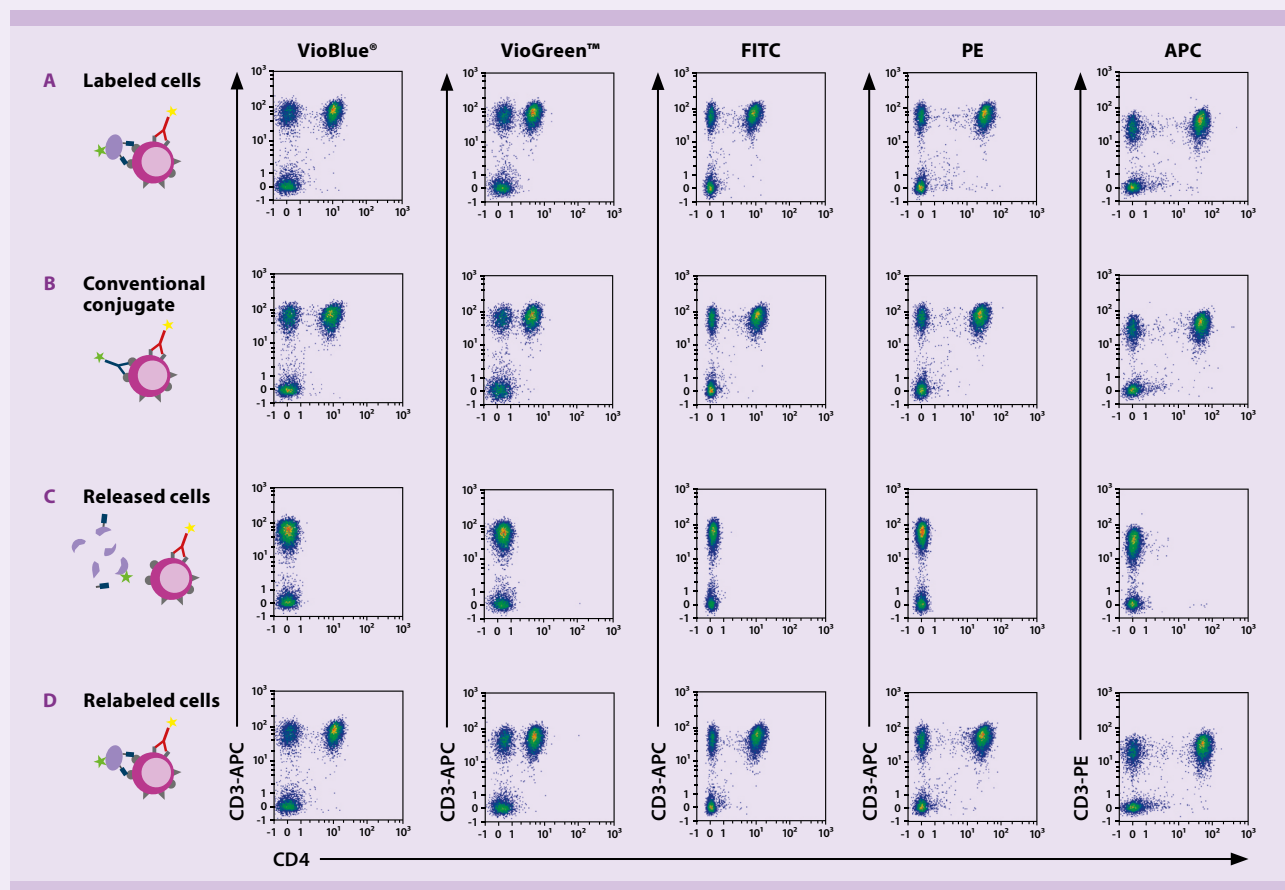


Figure 2: Staining and release performance of REAlease Antibodies. Human peripheral blood mononuclear cells (PBMCs) were stained with REAlease Releasable Antibodies (A) or conventional antibodies (B) targeting CD4 and conjugated to different fluorochromes. All samples were also counter-stained with conventional CD3 antibodies. REAlease Antibodies were efficiently released using the REAlease Release Reagent (C) and successfully relabeled (D) using the same REAlease Releasable Antibodies targeting CD4. All samples were analyzed by flow cytometry on the MACSQuant® X.

Applying REAlease® Fluorochrome Technology

Maximal flexibility for a wide range of applications

- Epitope of a marker becomes available again for downstream applications that require free epitopes (e.g., cell culture experiments and cell analysis)
- Fluorescence channel becomes available again for relabeling (e.g., microscopy applications)
- Sequential flow sorting cycles for the isolation of specific cell subsets

Multiparameter staining and release

As most target cell populations are defined by the expression of several antigens, an important requirement for their straight isolation by flow sorting is the possibility to label several markers simultaneously. REAlease® Technology enables reversible multiparameter cell labeling by allowing the reuse of individual fluorescence channels. To demonstrate the versatility of this technology, we developed releasable fluorochrome conjugates for several cell markers that can be used for multiparameter panel staining of, e.g., regulatory T (Treg) cells, or natural killer (NK) cells (fig. 3).

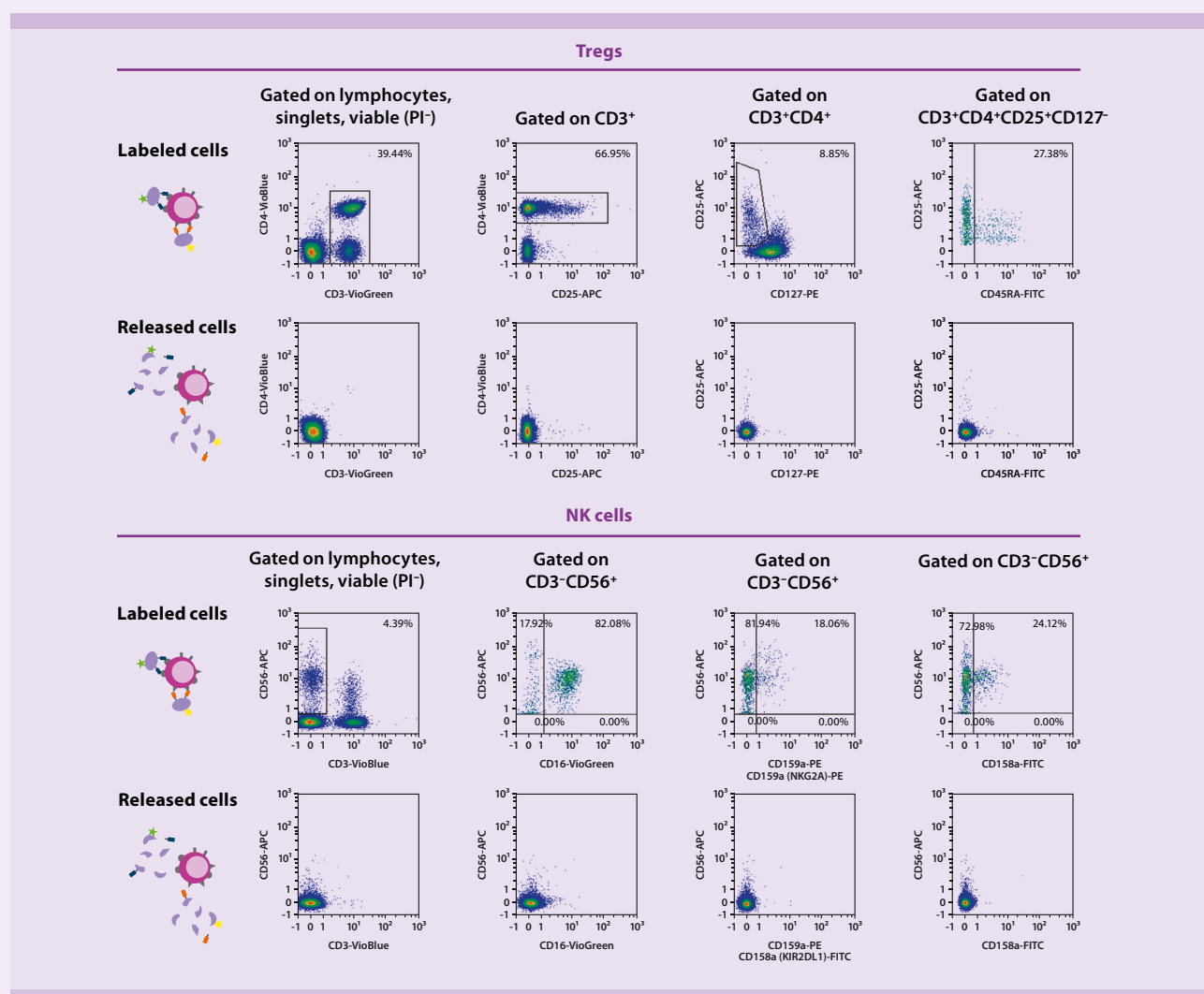


Figure 3: Multiparameter immunofluorescent staining and release. One PBMC sample was used as starting material for a five-parameter panel labeling including regulatory T cells, and natural killer (NK) cells. Labeling with releasable antibody fluorochrome conjugates resulted in efficient staining. Subsequent release using the REAlease Release Reagent yielded completely unlabeled cells.

Product	Order No.
REAlease CD4-FITC, human	130-112-071
REAlease CD4-PE, human	130-117-725
REAlease CD4-VioGreen, human	130-115-434

Find all available REAlease® Fluorochrome conjugated antibodies at
www.miltenyibiotec.com/realease-fluorochromes

Treg cell panel

REAlease CD3-VioGreen, human	130-115-972
REAlease CD4-VioBlue, human	130-118-942
REAlease CD25-APC, human	130-116-230
REAlease CD127-PE, human	130-116-559
REAlease CD45RA-FITC, human	130-112-079

Pan T cell panel

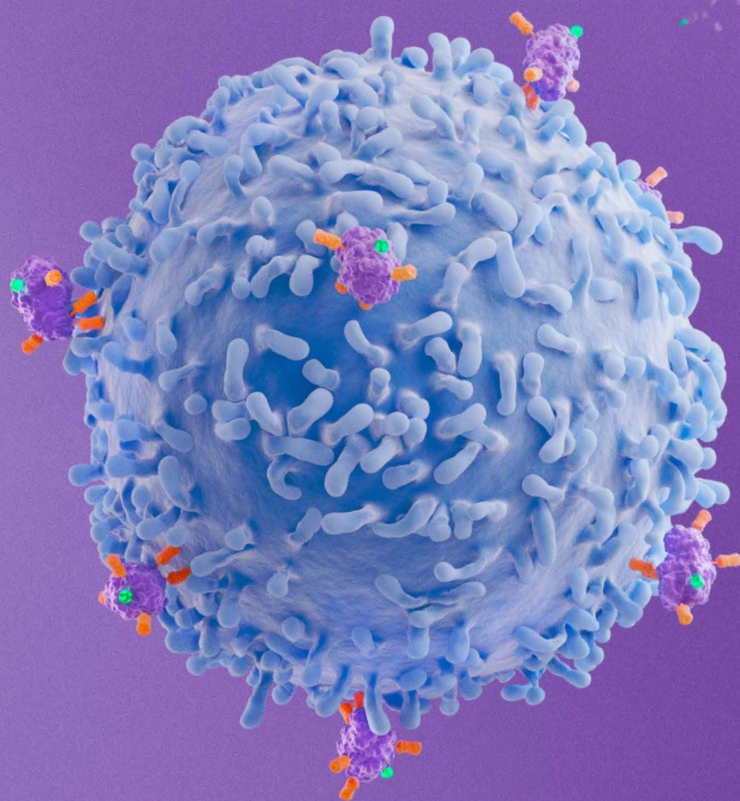
REAlease CD3-VioBlue, human	130-118-941
REAlease CD4-APC, human	130-112-089
REAlease CD8-VioGreen, human	130-115-197
REAlease CD62L-PE, human	130-117-728
REAlease CD45RA-FITC, human	130-112-079

NK cell panel

REAlease CD3-VioBlue, human	130-118-941
REAlease CD56-APC, human	130-116-548
REAlease CD16-VioGreen, human	130-115-975
REAlease CD159a-PE, human	130-118-926
REAlease CD158a-FITC, human	130-114-948

Other

REAlease Support Kit	130-120-675
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