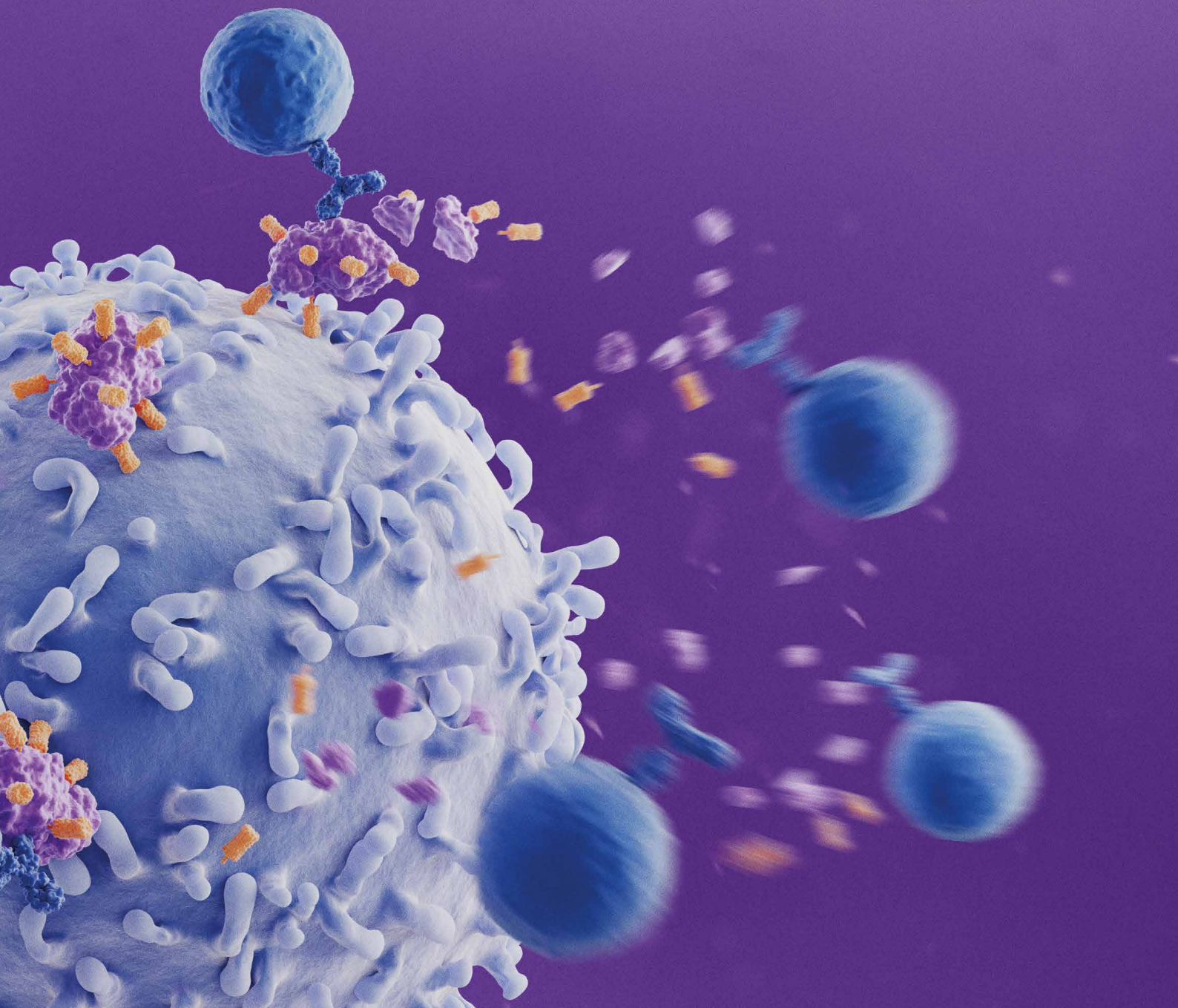
MACS

Miltenyi Biotec

REAlease™ Immunomagnetic Separation Technology

Reversible cell labeling for maximal flexibility



REAlease™ MicroBeads for reversible cell labeling

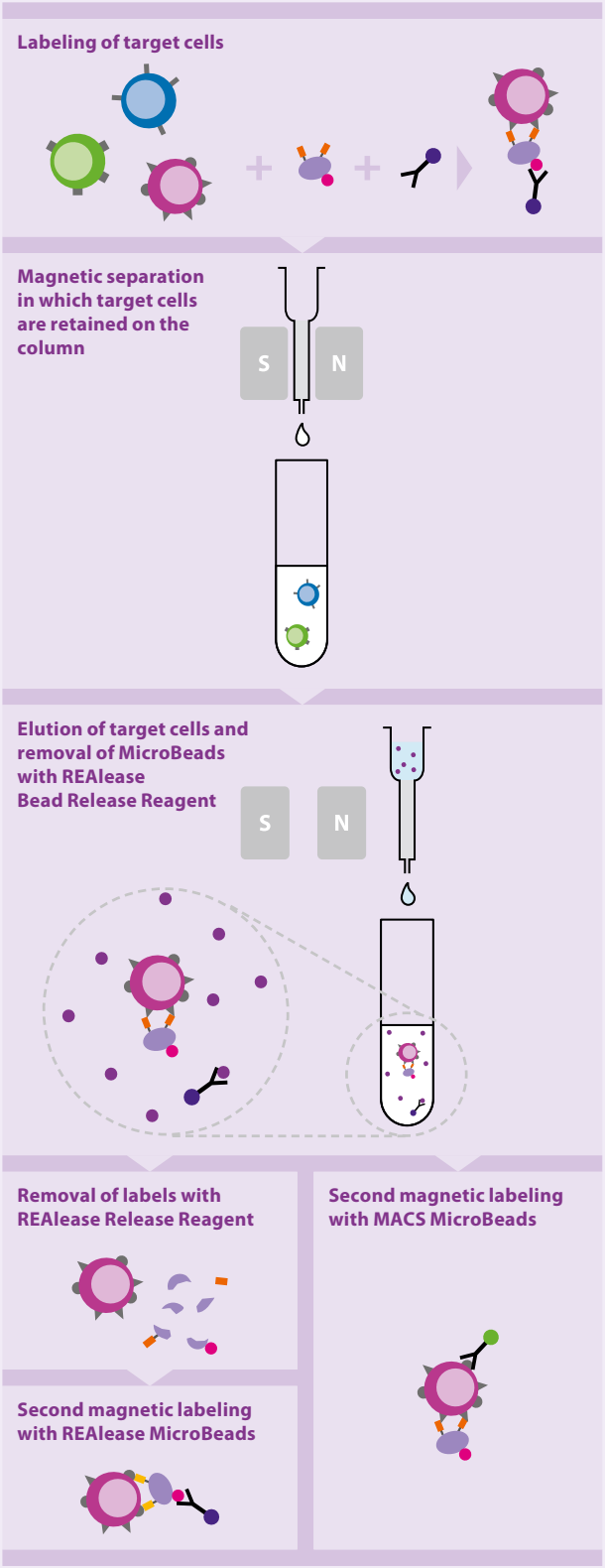
- The next step in flexibility for cell sorting
- Label-free cells for downstream applications
- Recombinantly engineered for reproducible results

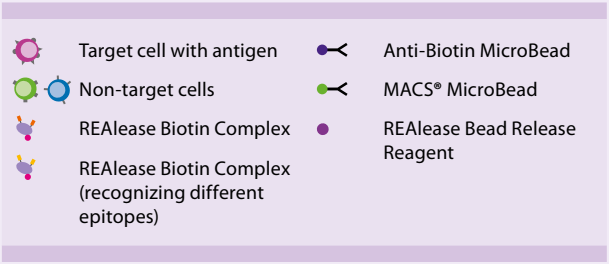
The basic principle

For certain applications, it is desirable to remove labeling from the isolated cells. Getting label-free cells does not have to be complicated. In contrast to cell surface labeling with whole antibodies, REAlease™ Technology relies on recombinantly engineered antibody fragments. The monomeric fragments were developed to have a low affinity for markers whereas multimerized as a REAlease Complex, they bind cell surface markers with high avidity. REAlease Technology controls the multimeric state of the antibody fragments: addition of the REAlease Release Reagent to the REAlease Complex leads to monomerization of the antibody fragments and spontaneous dissociation of the REAlease Complex from the cell surface (fig. 1). The result: label-free cells for maximal flexibility in cell isolation.

The unexpected opportunities

With REAlease Immunomagnetic Separation Technology, target cells are positively selected using an indirect magnetic cell labeling method (fig. 1). Following cell separation, MicroBeads and REAlease Biotin Complex are gently removed, leaving the cells bead- and label-free. MicroBead-free cells are suitable for consecutive magnetic labeling while completely label-free cells are suitable for any downstream application, as their markers are available for re-labeling.
The REAlease Complex is recombinantly engineered and produced to provide maximal lot-to-lot consistency, which ensures high reproducibility of your experimental results.





Magnetic isolation of pure cells

Magnetic cell separation using REAlease™ MicroBeads results in high purities of target cells comparable to our proven and trusted MACS® MicroBeads (fig. 2).

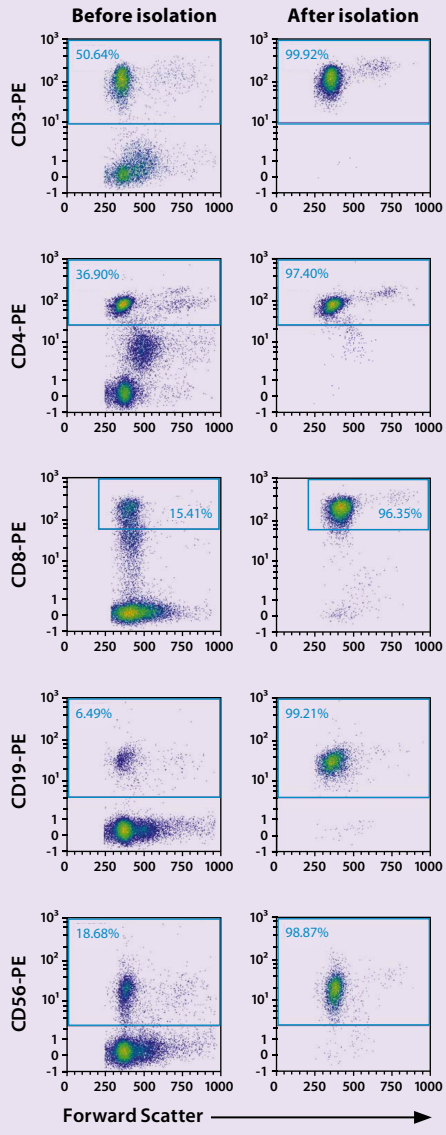


Figure 2: Isolation of highly pure target cells using REAlease MicroBeads. Before and after isolation, the cells were stained with the indicated fluorochrome-conjugated antibodies and analyzed on a MACSQuant® X. Isolated cells were free from MicroBeads and REAlease Biotin Complexes.

Efficient bead release from target cells

REAlease MicroBeads can be released from magnetically enriched target cells with an efficiency higher than 99% (table 1). This results in bead-free cells, ready for a second round of magnetic labeling.

Efficiency of bead release (%)		
	Mean	**SD**
CD3	99.6	0.27
CD4	99.8	0.09
CD8	99.6	0.15
CD19	99.5	0.19
CD56	99.4	0.29

Table 1: Efficiency of magnetic labeling removal. Following magnetic cell separation, the MicroBeads were released from the isolated cells using the REAlease Bead Release Reagent. Isolated cells were then re-applied to a second MACS Column. Efficiency of REAlease MicroBead release was calculated based on the ratio between the numbers of cells in the flow-through and the total number of cells applied to the second column.

Efficient label release from target cells

After releasing the REAlease™ Biotin Complex, the epitope of the respective marker becomes completely available again as shown in figure 3. This quick and gentle method is perfectly suited for any downstream application where label-free cells are essential.

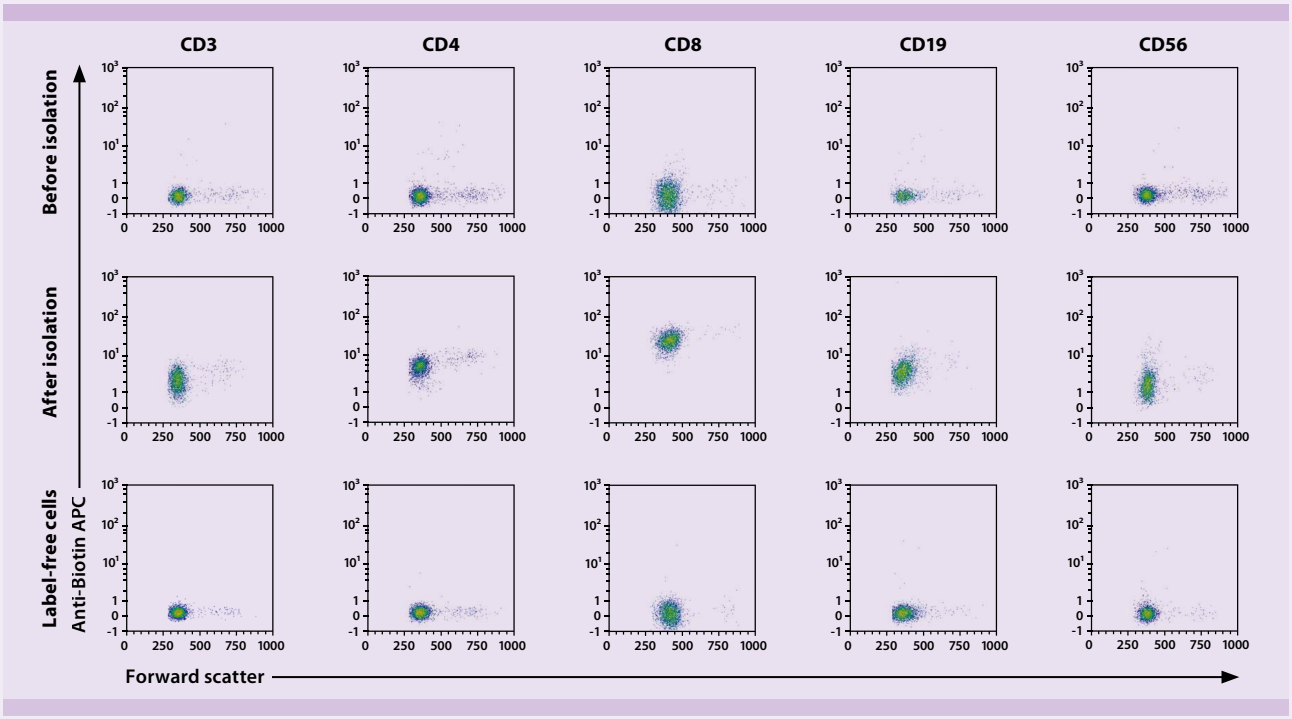


Figure 3: Analysis of label-free cells. To show that cells are free from labels, target cells were analyzed by labeling with Anti-Biotin-APC antibodies. Label-free cells were negative for biotin, similar to the non-labeled cells before separation demonstrating a highly efficient release of the REAlease Biotin Complex.

Applying REAlease™ Immunomagnetic Separation Technology

Maximal flexibility for applications

- Sequential positive selection steps with REAlease™ MicroBeads for the isolation of a target cell type based on multiple markers (Treg cells $CD4^+CD25^+$)
- Positive selection of a target cell type with REAlease MicroBeads followed by depletion of unwanted cells from this cell population using regular MACS® MicroBeads (fig. 4)
- Positive selection of a target cell type followed by positive selection of another target cell type from the first negative cell fraction (label-free $CD4^+$ and $CD8^+$ cells)

Product	Order No.
REAlease CD3 MicroBead Kit, human	130-117-038
REAlease CD4 MicroBead Kit, human	130-117-037
REAlease CD8 MicroBead Kit, human	130-117-036
REAlease CD19 MicroBead Kit, human	130-117-034
REAlease CD56 MicroBead Kit, human	130-117-033
MACS LS Columns	130-042-401
MACS MS Columns	130-042-201

NK cell isolation using REAlease MicroBeads

Highly pure NK ($CD56^+CD3^-$) cells can be isolated from PBMCs by enriching $CD56^+$ cells using REAlease CD56 MicroBeads and depleting unwanted $CD3^+$ cells with MACS CD3 MicroBeads (fig. 4).

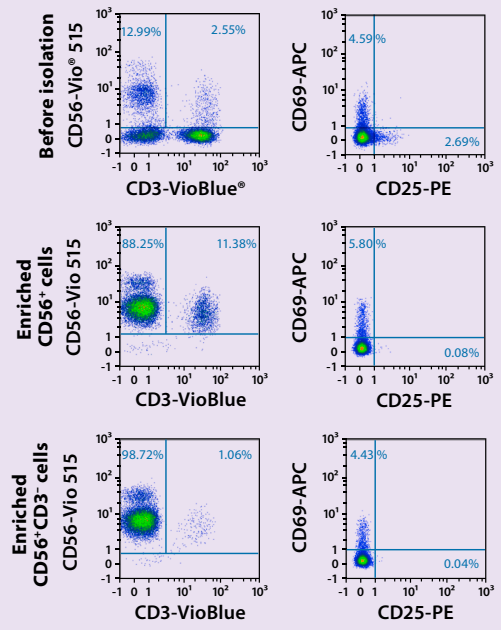
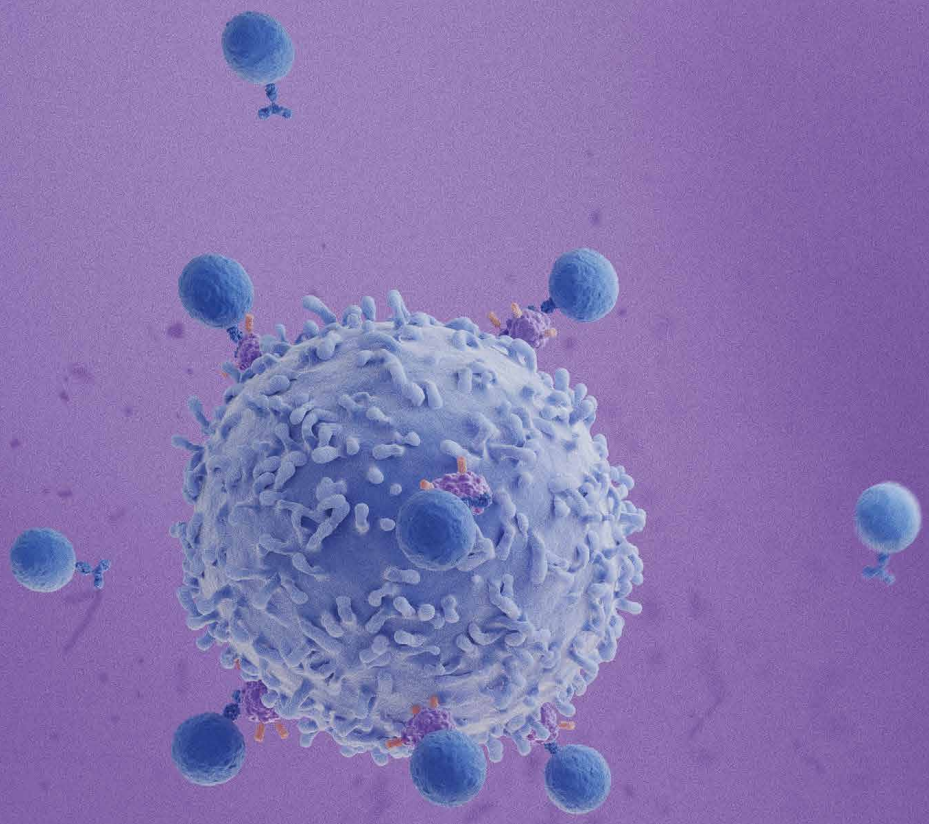


Figure 4: Isolation of $CD56^+CD3^-$ NK cells.
REAlease CD56 MicroBeads were used for the enrichment of $CD56^+$ cells from PBMCs. After removal of the REAlease MicroBeads, the $CD3^+$ cell fraction was depleted from the $CD56^+$ cell population with MACS CD3 MicroBeads to yield $CD56^+CD3^-$ NK cells with a high purity of 98%. No difference was observed in the levels of the activation markers CD69 and CD25 between the cells before and after isolation.

▶ miltenyibiotec.com/realease-microbeads







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