REAlease™ Immunomagnetic Separation Technology with reversible labeling for positive selection of leukocytes

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Introduction

Immunomagnetic enrichment of leukocytes is an important technique in both research and clinical applications. Accordingly, there is a continuous need for improved, reliable cell separation methods. Current enrichment strategies using magnetic labeling of the target cells allow for highly efficient isolation, but in some instances removal of residual cell surface labeling after isolation is of high interest. To this end, we have combined the benefits of positive selection by MACS® Technology, the proven state-of-the-art method for the isolation of functional, viable cells, with a novel technology enabling the removal of both superparamagnetic beads and antibody fragments. REAlease™ Technology provides an easy and fast solution for the highly specific isolation of unlabeled leukocytes directly from PBMCs. We present our latest results on cell separation with REAlease Technology based on key human immune cell markers, i.e., CD3, CD4, CD8, CD19, and CD56. The benefits of these releasable labels are demonstrated for the isolation of important subsets such as regulatory T (Treg) cells, $CD3^-CD56^+$ NK cells, and $CD4^+$ and $CD8^+$ cell populations from a single sample.

Methods

1 REAlease™ Technology – the principle

REAlease™ Technology relies on recombinantly engineered antibody fragments instead of antibodies to label specific cell surface markers. The antibody fragments are engineered to have low affinity for markers. However, when the fragments are multimerized as a complex they bind markers with high avidity. REAlease Technology can control the multimer/monomer state of the fragments allowing for a controlled release where monomerized antibody fragments dissociate from the cell surface, enabling users to obtain cells that are free from antibody fragments and magnetic label.

2 REAlease™ Technology – the procedure

First, the target cells in a PBMC population are labeled with a REAlease Complex that includes biotin and antibody fragments. Subsequently, the biotin molecules are labeled with superparamagnetic Anti-Biotin MicroBeads allowing magnetic isolation of target cells. To enrich the magnetically labeled cells, the cell suspension is applied onto a MACS Column placed in a magnetic field. Unlabeled non-target cells flow through. Then, the target cells are eluted from the column using the REAlease Bead Release Reagent, which concomitantly removes the MicroBeads from the cell (fig. 1). Using the REAlease Release Reagent, the REAlease Complex is disrupted resulting in monomerization of the antibody fragments and thus spontaneous dissociation of the fragments from the cell surface.

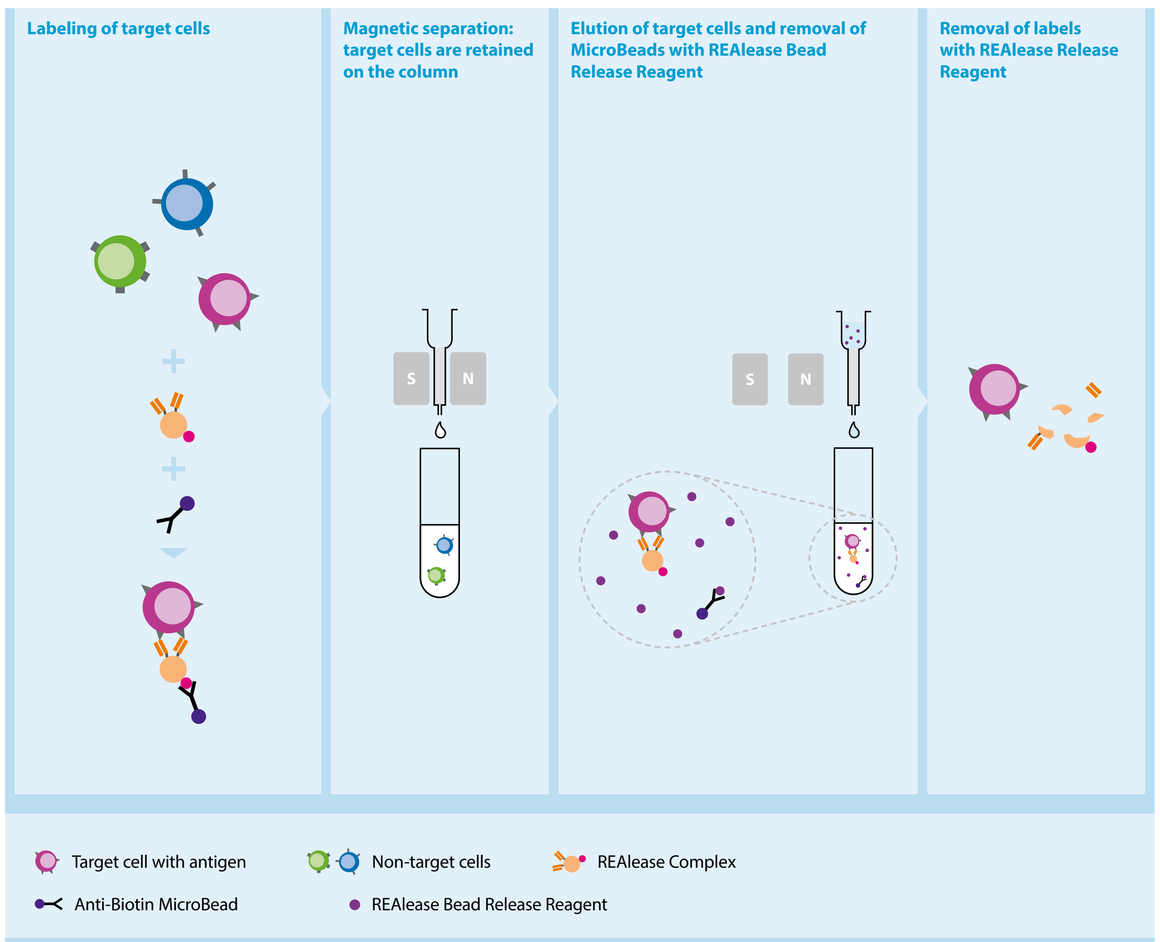

Figure 1

3 REAlease™ Technology – the options

REAlease™ Technology allows for the removal of either the MicroBeads or the entire labeling complex from the cells. Both options result in cells that are suited for a second magnetic labeling step. MicroBead removal is achieved during elution of the cells with REAlease Bead Release Reagent. To dissociate the REAlease Complex, the REAlease Release Reagent is added to the target cells (fig. 1). This results in label-free cells.

Results

1 REAlease™ Technology enables isolation of highly pure immune cell subtypes and efficient release of any labels

Separation based on REAlease™ Technology resulted in high purities of CD3, CD4, CD8, CD19, and CD56 cells. Mean purities were 95% for each marker and the recovery was higher than 90% (except for CD56: 80%).
After separation, the MicroBeads were released from the isolated cells with REAlease Bead Release Reagent. Bead release efficiency was greater than 99% for the five cell types. We also have shown that the antibody fragments were effectively removed from the cells after addition of REAlease Release Reagent by using an Anti-Biotin-APC antibody to analyze the cells by flow cytometry for residual labeling of biotinylated REAlease Complex. Directly after isolation, the cells showed staining of biotin, whereas cells after label release were negative for biotin similar to the non-labeled cells before separation (data not shown).

2 Isolation of $CD4^+CD25^+$ Treg cells with REAlease™ CD4 MicroBeads and MACS® CD25 MicroBeads

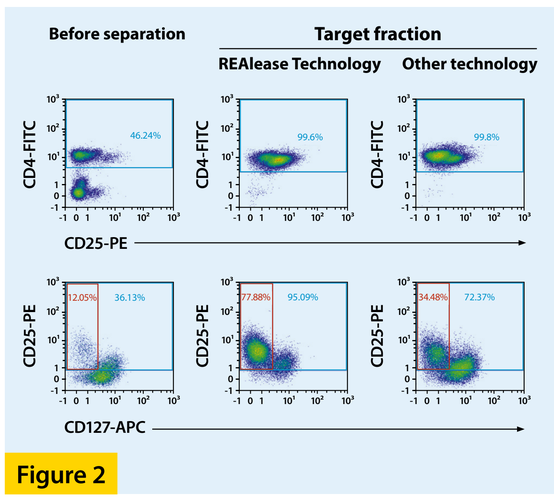

Figure 2

REAlease Technology allows for sequential positive selection steps based on multiple markers. In the experiment shown, $CD4^+CD25^+$ Treg cells, a rare cell population (~2%) in PBMCs, were isolated using REAlease Technology for the enrichment of $CD4^+$ T cells, followed by a second separation step with regular CD25 MicroBeads. The isolated Treg cells showed a high purity. We also compared the technology to another commercially available technique for the isolation of $CD4^+CD25^+$ cells with releasable magnetic labeling (fig. 2).

3 Isolation of $CD56^+CD3^-$ NK cells with REAlease™ CD56 MicroBeads and MACS® CD3 MicroBeads

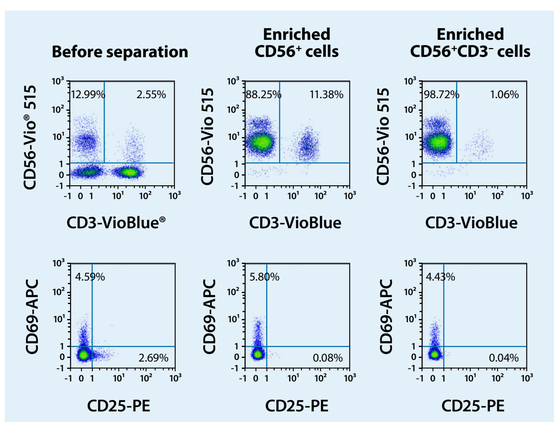

Figure 3

REAlease Technology also enables positive selection of a target cell type followed by depletion of unwanted cells from this cell population with regular MACS MicroBeads.
In the experiment shown, $CD56^+CD3^-$ NK cells were isolated from PBMCs. In the first step, REAlease CD56 MicroBeads were used for the enrichment of $CD56^+$ cells. 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%. We did not observe activation of the NK cells after isolation by monitoring CD69 and CD25 levels by flow cytometry.

4 Isolation of $CD4^+$ and $CD8^+$ label-free cells from a single sample

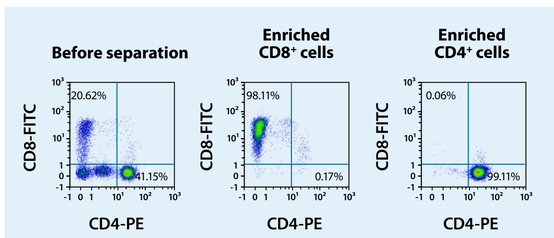

Figure 4

REAlease Technology allows for the positive selection of a target cell type followed by the positive selection of another target cell type from the first negative cell fraction. The use of REAlease MicroBeads for both separation steps results in two label-free cell populations.
In the experiment shown, $CD8^+$ cells were isolated from PBMCs using REAlease CD8 MicroBeads. The negative fraction of this separation step was used as starting material for the isolation of $CD4^+$ cells with REAlease CD4 MicroBeads. Both cell populations were isolated with yields higher than 80% and purities exceeding 98%.

Conclusion

The novel REAlease Technology combines two key features: highly specific cell isolation by positive selection and removal of any labels.

- After isolation of the cells the complete labeling complex (including REAlease Complex and superparamagnetic MicroBeads) can be released from the cell surface.
- Bead-free cells: suited for consecutive magnetic labeling.
- Label-free cells: the epitope of a marker with low abundance becomes available again.
- Recombinantly produced antibody fragments: lot-to-lot consistency allows for reproducible results.