# Reliable and effective positive isolation of CD45+ tumor-infiltrating leukocytes by MACS® and REAlease® Technologies

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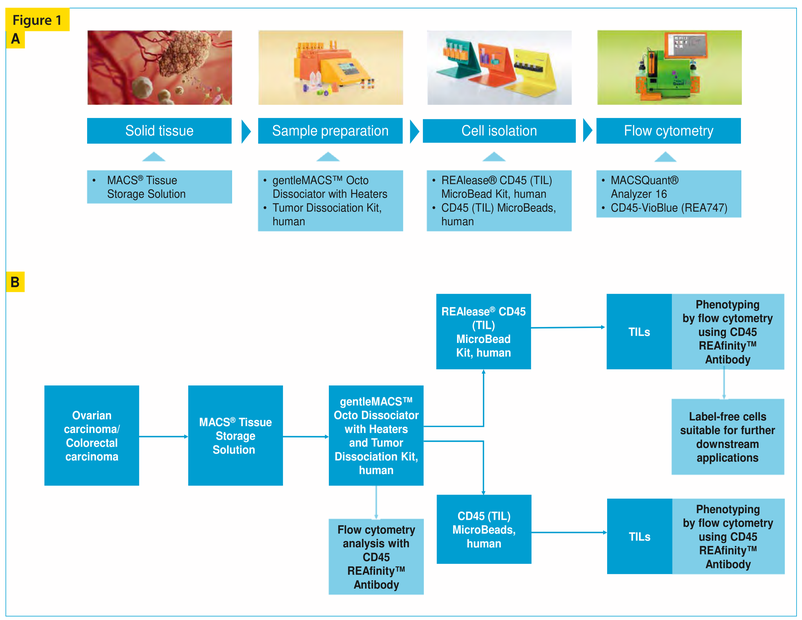
## Introduction

An in-depth characterization of tumor-infiltrating leukocytes (TILs) is crucial to further improve cancer immunotherapies. Since TILs generally constitute only a small subpopulation in solid tumors, they can be lost in the background noise of downstream analyses such as flow cytometry or single-cell sequencing. Therefore, improved methods for pre-enrichment of TILs are necessary to increase the sensitivity of such studies and save resources spent on the analysis of contaminating cell populations.
Current enrichment strategies using magnetic labeling of the target cells allow for highly efficient isolation. However, in some instances removal of residual cell surface labeling after isolation is of great importance. Therefore, 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 labeling. The REAlease® Technology provides an easy and fast solution for the highly specific isolation of unlabeled TILs, which can be used to isolate further subpopulations of interest.

## Materials and methods

We have established a reliable workflow combining automated tissue dissociation with positive selection of CD45+ TILs from human tumors as well as humanized mouse models. Human tumor samples were stored and shipped in MACS Tissue Storage Solution, maintaining cell viability and phenotype for up to 48 hours after collection. Tumor dissociation was automated using the gentleMACS™ Octo Dissociator with Heaters and optimized for epitope preservation to overcome bias in immunophenotyping caused by dissociation with aggressive or impure enzymes.
The direct isolation of TILs by MACS Technology was optimized based on a CD45-specific enrichment reagent, taking into account the characteristics of solid tumors, such as the presence of debris and dead cells. Furthermore, we developed an isolation strategy based on REAlease Technology, providing an easy and fast solution for the highly specific isolation of label-free TILs.
An overview of the workflow is shown in figure 1.

Figure 1

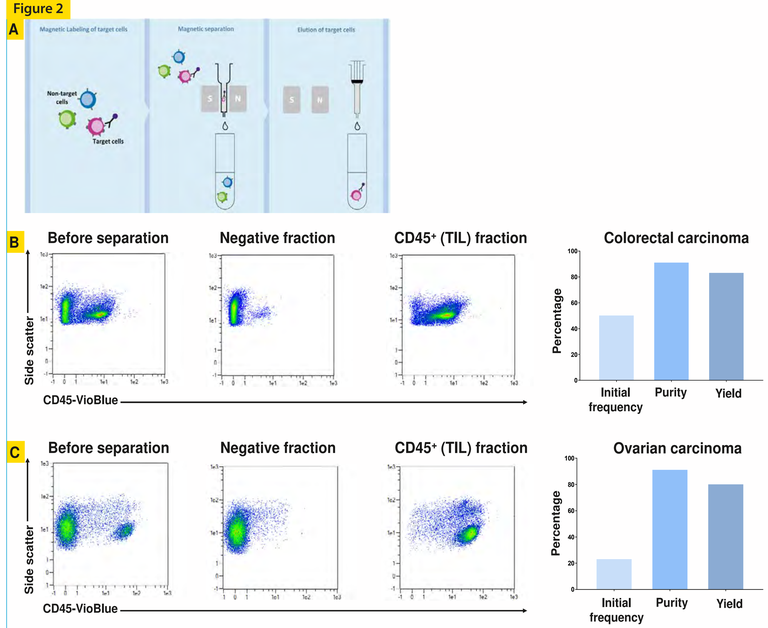



## Results

### 1 Isolation of TILs using MACS® Technology

We have developed a new human CD45-specific reagent for the enrichment of CD45-expressing TILs based on MACS® Technology. To validate the separation, we used freshly dissected human colorectal and ovarian carcinomas, which were stored and shipped overnight in MACS Tissue Storage Solution. After dissociation of the tumor tissue using the gentleMACS Octo Dissociator with Heaters and the corresponding Tumor Dissociation Kit, CD45+ target cells were isolated using the new CD45 (TIL) MicroBeads, human (fig. 2A). The colorectal carcinoma showed an initial frequency of TILs among living cells of 51%. MACS Technology–based isolation of CD45-expressing TILs resulted in a purity of 92% and a yield of 80% (fig. 2B).
TIL isolation from a human ovarian carcinoma with an initial frequency of 23% led to a purity of 90% and a yield of 80% (fig. 2C).

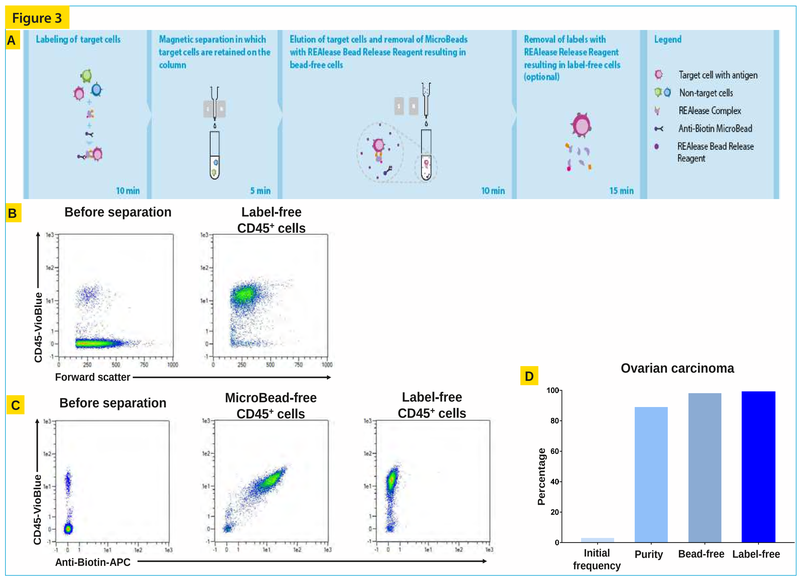
Figure 2




### 2 REAlease® Technology enables isolation of label-free TILs

To allow for the isolation of label-free TILs, we developed an enrichment strategy based on REAlease® Technology (fig. 3A). Similar to the approach presented above, this method is independent of the tumor entity. Even from tumors with low levels of TIL infiltration, e.g., an ovarian carcinoma with an initial TIL frequency of 4%, TILs could be enriched to a purity of 90% (fig. 3B, D).
After bead release, more than 99% of the cells showed no residual magnetic labeling (data not shown). Hence the isolated TILs can be used for a second round of magnetic isolation to enrich subpopulations.
Additionally, we demonstrated that the biotin-conjugated REAlease Complex was effectively removed from the target cells as well, making them non-distinguishable from cells before labeling (fig. 3C). As REAlease Technology enables the removal of all labels, the epitopes used for isolation are completely available for relabeling and can be further used for any downstream application.

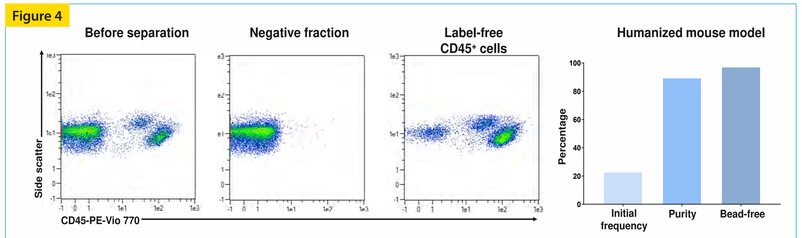
Figure 3




### 3 Isolation of human CD45-positive cells from humanized mouse models

The use of humanized mouse models is crucial for the evaluation of immunotherapeutic approaches. However, the analysis of human immune cells is frequently hampered because the human cells are usually outnumbered by mouse cells. The REAlease CD45 (TIL) MicroBead Kit, human allowed us to isolate human CAR T cells to high purities from the bone marrow of humanized NSG mice (fig. 4).

Figure 4




## Conclusion

- We established a reliable workflow for the dissociation of solid tumors and subsequent isolation of TILs within 90 minutes.
- Positive isolation of TILs based on the standard MACS Technology and REAlease Technology was highly efficient and reproducible.
- REAlease Technology enables effective removal of all labels from the target cells, which in turn allows for a second round of magnetic labeling to isolate subpopulations.