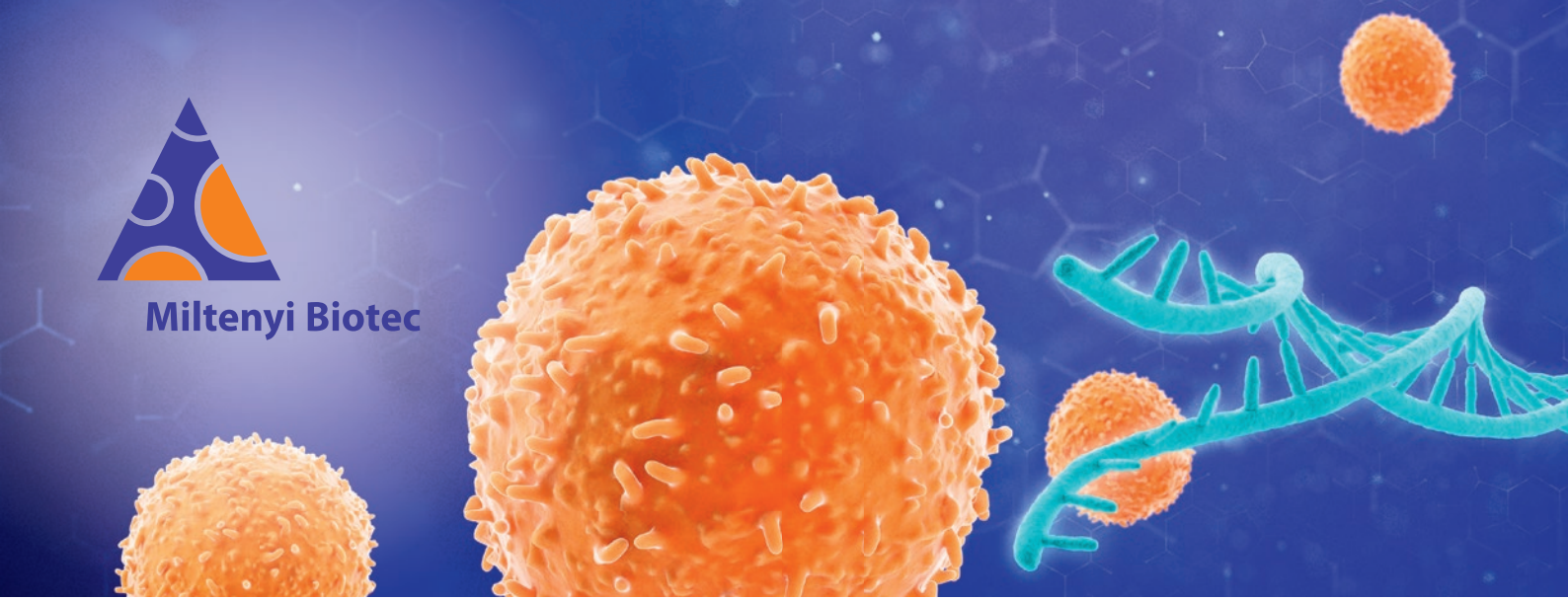

Increase the viability of your samples with the Dead Cell Removal Kit

Background

The presence of dead cells in cell suspensions can have a negative impact in many downstream applications, such as magnetic cell separation, cell sorting, cell culture, or single-cell genomic analysis. By removing dead cells, the efficiency of magnetic cell separations and cell cultivation is improved, cell sorting time is reduced, and recovery rates are increased when performing single-cell gene expression analysis. Therefore, the efficient removal of dead cells is an essential step to improve the quality of your samples and to get reliable results.

The Dead Cell Removal Kit enables the efficient magnetic depletion of dead cells from cell suspensions containing robust cells, such as fibroblasts, epithelial cells, immune cells, or tumor cells*. In addition, when combined with the autoMACS® Pro Separator, the process of dead cell removal can be performed in an automated way by just pressing one button.

* For cell suspensions containing fragile cells, such as adult neurons, adult cardiomyocytes, or hepatocytes we recommend using the Debris Removal Solution (130-109-398).

Application data

Successful increase of cell viability of poor-quality samples
Samples of peripheral blood mononuclear cells (PBMC) with different rates of cell viability were subjected to dead cell removal using the Dead Cell Removal Kit, either following the manual protocol or the automated protocol using the autoMACS Pro Separator. In all cases cell viabilities were increased to over 85% independently of using the manual or the automated protocol (fig. 1).

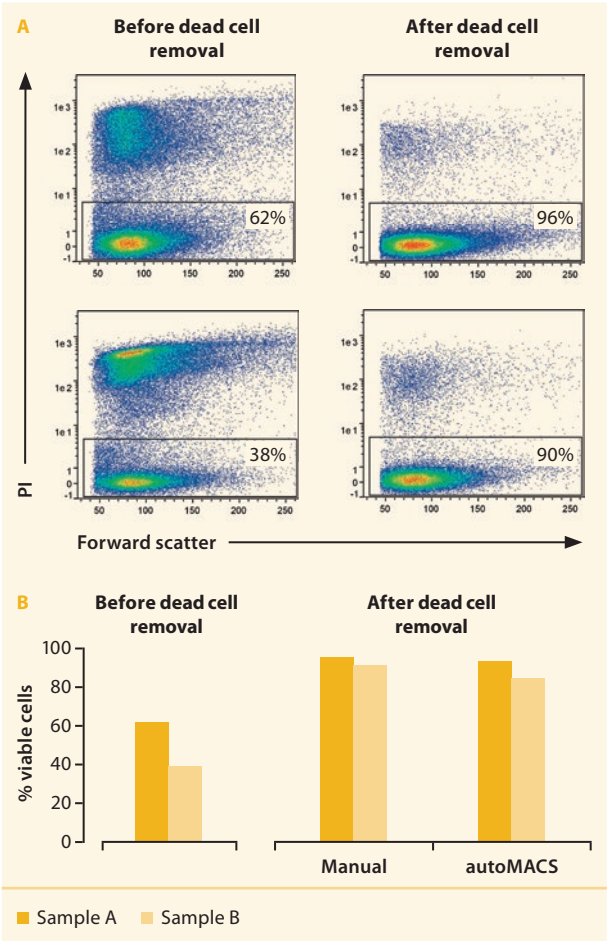

Figure 1: Removal of dead cells from PBMC samples with different cell viability rates. (A) Exemplary dot plots showing the analysis of cell viability of samples before and after dead cell removal. Cell viability was determined by propidium iodide (PI) staining and analyzed by flow cytometry. Dead cells are defined as PI-positive cells and viable cells as PI-negative. Percentages shown correspond to viable cells. (B) Comparison of dead cell removal performed following either the manual protocol or the automated protocol using the autoMACS Pro Separator.

Effective isolation of T cells from cryopreserved tumor samples after dead cell removal

A cryopreserved dissociated tumor sample was thawed and filtered using a MACS® SmartStrainer (70 µm). Cell viability in the thawed sample was below 30%. After removing dead cells with the Dead Cell Removal Kit, the viability rate was increased to 83% (fig. 2A). Subsequently, T cells were magnetically isolated using the REAlease® CD4/CD8 (TIL) MicroBead Kit, human to a purity of 80% (fig. 2B).

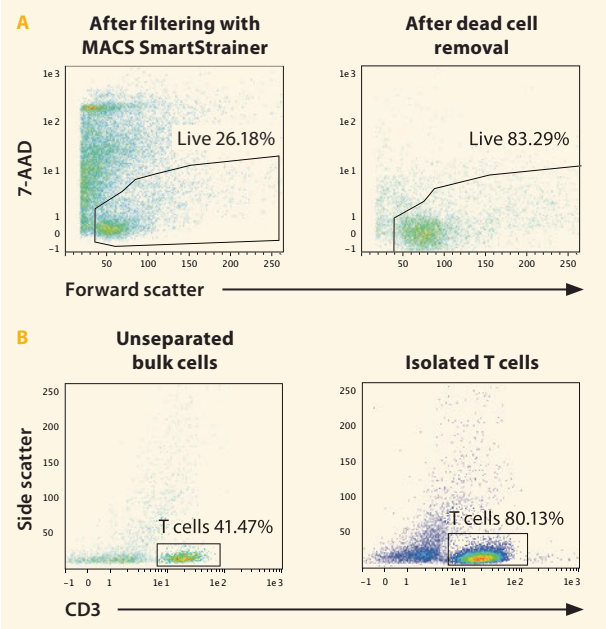

Figure 2: High purities of isolated CD4/CD8 T cells from human tumors after removal of dead cells. (A) Removal of dead cells from tumor samples after thawing. Cell viability was determined by 7-Aminoactinomycin D (7-AAD) staining analyzed by flow cytometry. (B) Flow cytometry analysis of CD4/CD8 T cells after dead cell removal and cell isolation.

Clean up of tumor samples improves the quality of single-cell genomic analysis

Single-cell suspensions from mouse tumors were obtained and subjected to various clean up steps prior to single-cell genomic analysis. To evaluate the quality of the samples, an aliquot of the cells was collected after each clean up step and single-cell gene expression analysis was performed. The quality of the sequencing results was evaluated using standard single-cell sequencing parameters, such as library cleanliness, library complexity, and cell recovery. The number of recovered cells indicates the number of cells applied to the 10x Genomics Chromium™ Platform that generated functional libraries, as cell recovery can be up to 65% of cells applied according to the manufacturer. Removal of dead cells in the final clean up step improved library cleanliness and library complexity (data not shown) and helped to increase the cell recovery rate (fig. 3).

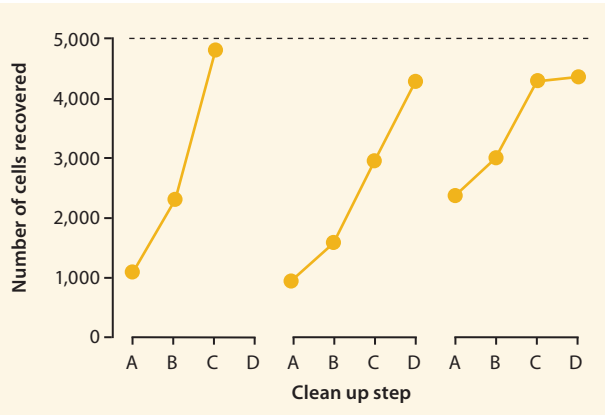

Figure 3: Impact of sample clean up in single-cell RNA sequencing of mouse colon tumors. Tumors were dissociated using the gentleMACS™ Octo Dissociator with Heaters and the Tumor Dissociation Kit, mouse and single-cell suspensions were filtered using a 70 µm MACS SmartStrainer (step A). Subsequently, red blood cells were removed with Red Blood Cell Lysis Solution (step B). Depending on the starting frequencies of viable cells in the samples, dead cells were removed either once (step C) or twice (step D) using the Dead Cell Removal Kit. Cell aliquots were taken at each step and single-cell RNA sequencing libraries were generated using the Chromium™ Single Cell v2 Gene Expression Solution (10x Genomics) according to the manufacturer's instructions. Libraries generated were sequenced using an Illumina® HiSeq® 4000 (Illumina), and data was analyzedd using the Cell Ranger™ software (10x Genomics). Cell recoveries are shown for three tumor replicates. The aimed number of recovered cells was 5,000 cells.

LEARN MORE

Learn more about the impact of sample preparation on genomic data quality at

▶ **miltenyibiotec.com/GenomicsAppNote**

Conclusions

- Our Dead Cell Removal Kit achieves the efficient removal of dead cells from different types of samples, both by manual or automated separation using the autoMACS® Pro Separator.
- Removal of dead cells from samples prior to single-cell genomic analysis improves library cleanliness and library complexity, and most importantly, increases the cell recovery rate, leading to higher genomic data quality.

Product	Order no.
Dead Cell Removal Kit	130-090-101
autoMACS Pro Separator Starter Kit	130-092-545
REAlease CD4/CD8 (TIL) MicroBead Kit, human	130-121-561
MACS SmartStrainers (70 µm)	130-098-462
gentleMACS Octo Dissociator with Heaters	130-096-427
Tumor Dissociation Kit, mouse	130-096-730
Red Blood Cell Lysis Solution (10×)	130-094-183

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