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Optimal storage of murine tumors in MACS® Tissue Storage Solution

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Background

The storage of fresh organs and solid tissue samples is often required in research projects when shipping samples to and from external sources, when samples need to be collected at different times, or simply when samples cannot be processed all at once. In these cases, short-term storage allows full flexibility for further processing of the tissues within a few days. For the analysis of viable cells from the stored tissues, it is essential to avoid necrosis and apoptosis during the storage period. Additionally, cell activation must be prevented, as well as changes in pluripotency of cells to preserve the physiological and functional status of the cells. A common storage method is to freeze tissue samples, but this frequently leads to high cell death after thawing and is a challenge for shipping.

To overcome all these issues, we have specifically developed the MACS Tissue Storage Solution, which allows the optimal storage of fresh solid tissues for at least 48 hours at 2–8 °C. Within this storage period, cell viability, functional and activation status of cells are preserved, and induction of cellular stress-related genes is prevented. MACS Tissue Storage Solution has been tested with a variety of human and murine tissues including tumor, lung, spleen, brain, and skeletal muscle.

Here, we show how MACS Tissue Storage Solution was used to preserve murine tumors for up to 48 hours. The cell viability and composition of tumor infiltrating leukocyte (TIL) populations were analyzed along with the expression of cellular stress-related genes at different points of time in storage.

Materials and methods

Storage of tumors

Murine CT26 tumors were collected and cut into pieces of similar size. An equal number of tumor pieces were distributed into two 15 mL tubes containing 5 mL of MACS Tissue Storage Solution and stored at 2–8 °C for 24 and 48 hours. Fresh and stored tumor pieces were processed the same way at each indicated time point.

Tumor dissociation

Tumors were dissociated using the gentleMACS™ Octo Dissociator with Heaters and the Tumor Dissociation Kit, mouse according to the instructions provided in the corresponding data sheet. ([The data sheet is available for direct download on the product page](#)). After dissociation, the single-cell suspensions obtained were filtered using MACS SmartStrainers (70 µm). An aliquot of the cells was used for flow cytometric analysis and the rest of the cells were stored in RLT buffer (Qiagen) until further processing for gene expression analysis.

Flow cytometric analysis

TIL populations were analyzed by flow cytometry using the following anti-mouse REAfinity® Antibodies: CD45 (REA737), CD3 (REA614), CD25 (REA568), CD69 (REA937), CD4 (REA604), CD8a (REA601), CD19 (REA749), CD80 (REA983) and CD86 (REA1190). Samples were analyzed using a MACSQuant® Analyzer 10. Propidium iodide was used to exclude dead cells. After dead cell exclusion, TIL populations were analyzed from gated CD45⁺ viable cells.

Gene expression analysis

RNA was isolated from fresh and stored cells using the RNeasy® Mini Kit (Qiagen). cDNA was produced using the RT2® First Strand Kit (Qiagen), and gene expression was analyzed by using the RT2 Profiler® PCR Array (Stress and Toxicity PathwayFinder®, mouse) and GeneGlobe® Analysis tool, both from Qiagen.

Results

MACS Tissue Storage Solution preserves cell viability and composition of TIL populations for up to 48 h in storage

CT26 tumors were processed on the same day of collection or after 24 and 48 hours of storage in MACS Tissue Storage Solution. After dissociation, total cell viability and composition of TILs was analyzed by flow cytometry. Cell viability was over 85% in both fresh and stored samples (fig. 1A). In addition, the composition of TILs was also preserved over time (fig. 1B). The activation status of the cells, observed by analyzing the expression of the activation markers CD25, CD69, CD80 and CD86 in specific TIL populations, was also found to be preserved.

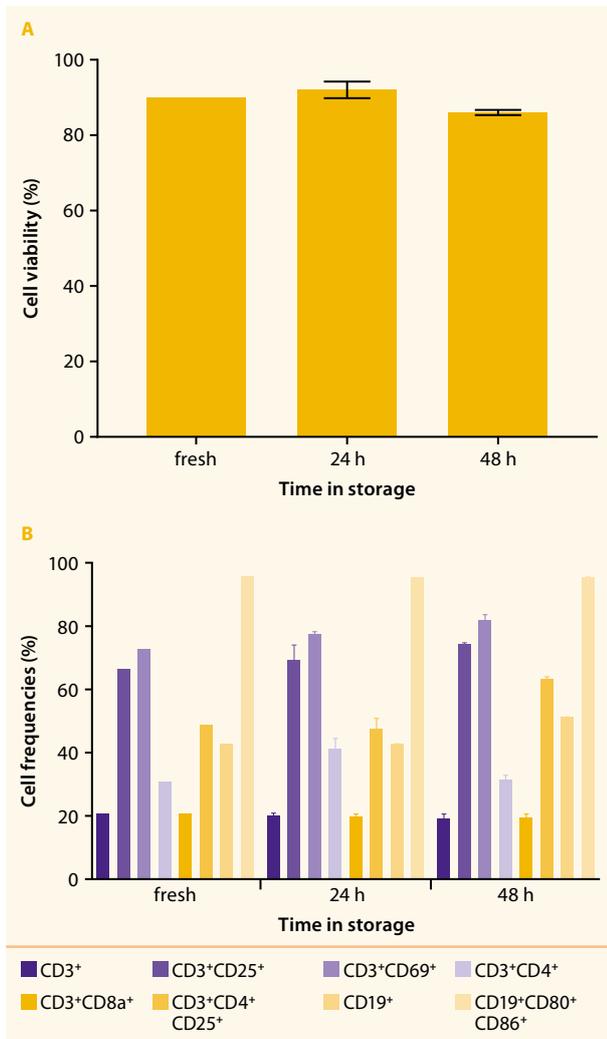


Figure 1: CT26 mouse tumors were stored at 4 °C in MACS Tissue Storage Solution for 24 or 48 h. After dissociation, total cell viability (A) and TIL composition (B) were analyzed by flow cytometry. Cell frequencies are referred to viable CD45⁺ cells. Additionally, frequencies of CD3⁺ and CD19⁺ cell subsets refer to corresponding parental CD3⁺ and CD19⁺ populations respectively.

Expression of cellular stress-related genes is prevented during storage in MACS Tissue Storage Solution

A major concern for molecular analysis is that storage conditions induce cellular stress and changes in gene expression. This was investigated by analyzing the expression of genes related to cellular stress pathways. Gene expression of the stored samples was compared to fresh samples. No major changes in expression of cellular stress-related genes were observed in the cells after 24 or 48 hours of storage, and gene expression was comparable to that of cells from fresh samples (fig. 2).

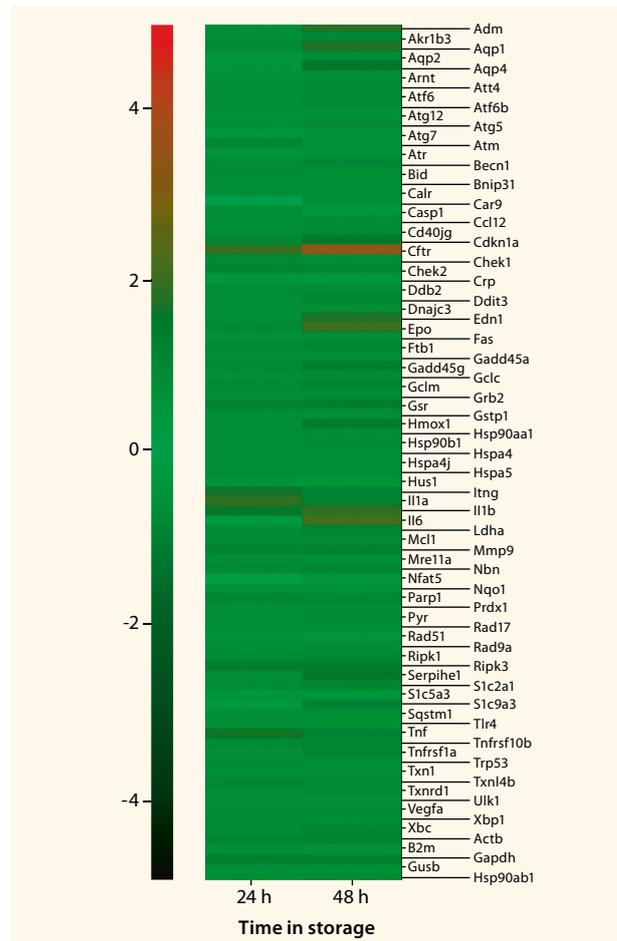


Figure 2: Expression of cellular stress-related genes in cells from fresh and stored tumors. RNA was isolated from fresh and stored cells from CT26 tumors and expression of cellular stress-related genes was analyzed. The heat map shows the fold-change of expression rates compared to fresh tissue.

Conclusions

- MACS Tissue Storage Solution represents a robust and convenient solution for short-term storage of different tissue samples and organs.
- Cell viability, cellular composition, and activation status of cells are well preserved for at least 48 hours.
- Lastly, MACS Tissue Storage Solution prevents cellular stress responses, since changes in cellular stress-related gene expression levels were kept to a minimum compared to fresh sample material.

Product	Order no.
MACS Tissue Storage Solution	130-100-008
gentleMACS Octo Dissociator with Heaters	130-096-427
Tumor Dissociation Kit, mouse	130-096-730
MACS SmartStrainers (70 µm)	130-098-462
MACSQuant Analyzer 10	130-096-343

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