Miltenyi Biotec

Efficient serum-free cryopreservation of primary cells and tumors with MACS® Freezing Solution

Background

Cryopreservation of cells and solid tissues is a common lab practice for long-term preservation and delayed use of samples. This procedure is fairly straightforward; cell or tissue samples are slowly frozen in a cryopreservation medium, stored in liquid nitrogen, and thawed quickly when they are needed. The cryopreservation medium creates a supportive environment for the cells that prevents the formation of ice crystals, which would otherwise damage the cell membrane, and enables the cells and tissues to recover after thawing. Therefore, the composition of the cryopreservation medium can have a big impact in the recovery of viable cells after thawing. This is especially true for primary cells, which are rather sensitive and demand a more supportive medium to survive the freeze-thaw process. The addition of serum to cryopreservation medium helps to improve the survival rate of the cells after thawing, but it comes with the risk of inconsistent results due to lot-to-lot variations, as well as induction of cell activation that may lead to unwanted background effects during functional assays.

We have developed the MACS Freezing Solution with consideration of the special needs of primary cells and tissues during freeze-thawing. This is a chemically defined, serum-free, and animal component–free cryopreservation medium that is ready to use. MACS Freezing Solution allows for the fast preparation of the samples for freezing and ensures the preservation of cell viability and cellular composition of primary cell suspensions and solid tissues after thawing.

Here we show how the MACS Freezing Solution was used to cryopreserve peripheral blood mononuclear cells (PBMCs), mouse tumors, and tumor cell suspensions. The samples were stored for two weeks in liquid nitrogen and cell recovery, cell viability, and cellular composition were analyzed afterwards.

Materials and methods

Tumor dissociation

CT26 mouse tumors were dissociated into single-cell suspensions using the Tumor Dissociation Kit, mouse in combination with the gentleMACS™ Octo Dissociator with Heaters, either fresh or after two weeks of cryopreservation.

Cryopreservation of samples

1. PBMCs: PBMCs from three different donors were split into two fractions for cryopreservation. One fraction was cryopreserved using RPMI containing 20% FCS and 10% DMSO, and the other fraction using MACS Freezing Solution. PBMCs from both fractions were preserved at a concentration of 10^7 cells/mL. Fresh PBMCs from each donor were analyzed as a control.

2. Tumors: CT26 mouse tumors (three biological replicates) were cut into small pieces, pooled, and split into two groups for cryopreservation. One group was cryopreserved using RPMI containing 20% FCS and 10% DMSO, and the other using MACS Freezing Solution. Up to 200 mg of tumor pieces were immersed per milliliter of each cryopreservation medium. Fresh tumor pieces were dissociated and analyzed as a control.

3. Primary tumor cell suspensions: Tumor cell suspensions from CT26 mouse tumors (three biological replicates) were split into two fractions for cryopreservation. One fraction was cryopreserved using RPMI containing 20% FCS and 10% DMSO, and the other using MACS Freezing Solution. Cell suspensions from both fractions were preserved at a concentration of 10^7 cells/mL. Fresh tumor cell suspensions were analyzed as a control.

In all cases, the samples were aliquoted in cryopreservation vials, placed in a freezing container and immediately stored at –80 °C. After 24 h, the vials were transferred to liquid nitrogen and stored for two weeks.

Sample thawing
All cryopreserved samples were thawed according to the protocol indicated in the data sheet of the MACS Freezing Solution.

Flow cytometric analysis
Cell count, cell viability, and the cellular composition of all fresh and cryopreserved samples were analyzed by flow cytometry. Cell count and cell viability were determined by volumetric pipetting and propidium iodide staining. The PBMC composition was analyzed using the 8-color Immunophenotyping Kit, human, and the tumor cell composition was analyzed using REAfinity™ Recombinant Antibodies against cell-specific markers: CD45, CD44, CD31, CD90.2, CD11b, CD3, Ly6G, and NKp46. All flow cytometric analyses were performed using a MACSQuant® Analyzer 10.

Results

MACS Freezing Solution ensures the high preservation of cell viability and cell recovery after thawing
The viability of PBMCs and tumor cell suspensions cryopreserved in MACS Freezing Solution was well preserved after thawing, showing comparable results to those samples cryopreserved in serum-containing media (figs. 1A, 1B). The viability of the cells generated from the tumors after thawing and dissociation was slightly decreased compared to the fresh tumors, but comparable results were obtained with both serum-free and serum-containing media (fig. 1C). Cell recoveries were calculated as the frequency of total viable cells recovered after thawing with reference to the initial number of fresh cells that were cryopreserved. The recovery of PBMCs after thawing was close to 60% for both cryopreservation media, while the recovery of the tumor cell suspensions after thawing reached values of around 80%, with a tendency of higher recoveries for the tumor cell suspensions cryopreserved in MACS Freezing Solution (figs. 2A, 2B). A similar tendency of higher yields of viable cells obtained after thawing and dissociation was observed for the tumors cryopreserved in MACS Freezing Solution (fig. 2C). However, higher numbers of total viable cells were obtained after dissociation of fresh tumors than from freeze-thaw tumors (data not shown).

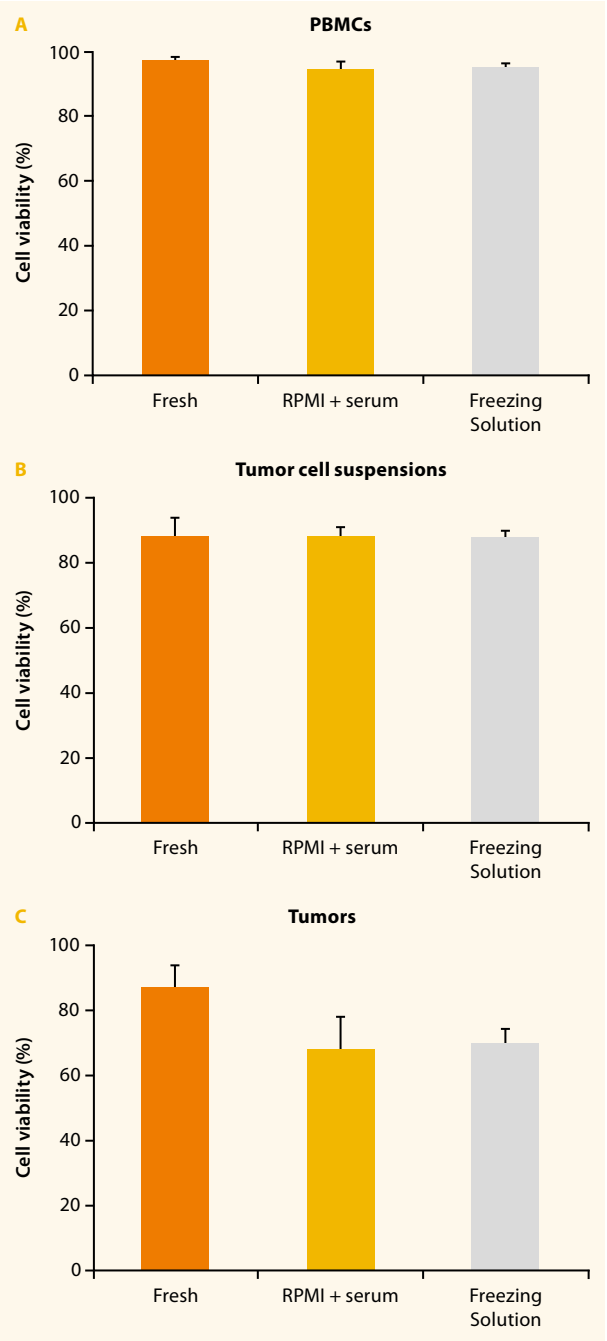

Figure 1: Cell viability of cryopreserved primary cells and tumors after thawing. PBMCs (A), tumor cell suspensions (B), and tumor samples (C) were cryopreserved either using a serum-containing medium or the serum-free MACS Freezing Solution. Cell viability was analyzed by flow cytometry after thawing of the cryopreserved samples. The thawed tumor samples were dissociated prior to the analysis. The cell viability of the corresponding fresh samples is shown as a control.

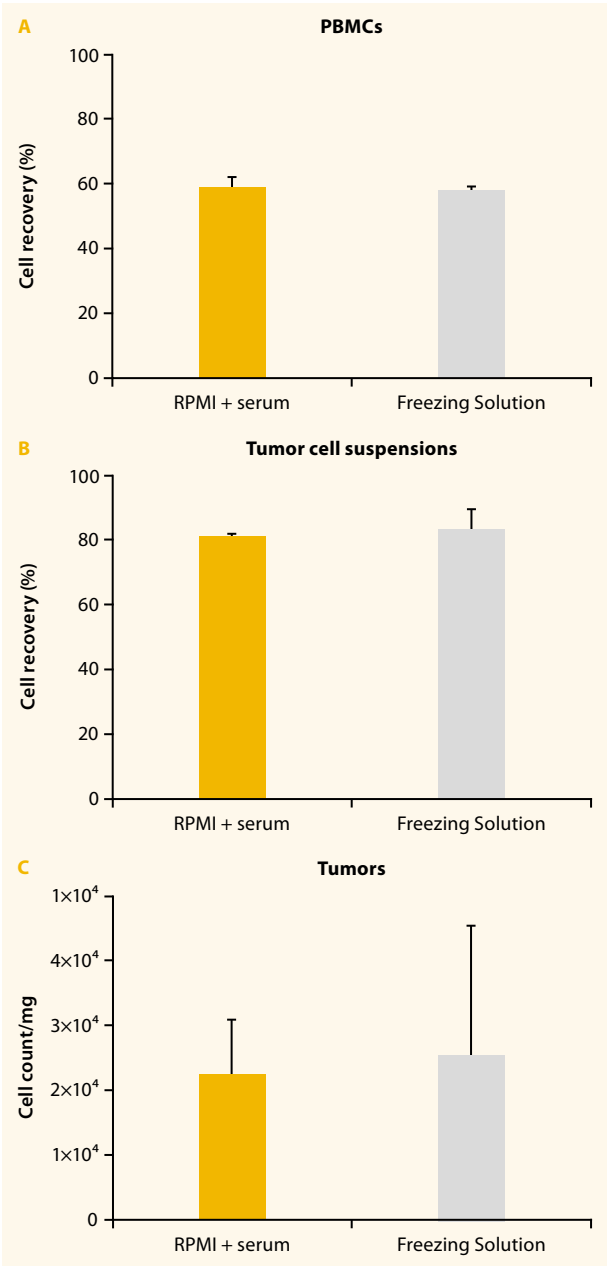

Figure 2: Recovery of cryopreserved primary cells after thawing. PBMCs, tumor cell suspensions, and tumor samples were cryopreserved either using a serum-containing medium or the serum-free MACS Freezing Solution. The recovery of PBMCs (A) and tumor cell suspensions (B) was calculated as the frequency of total viable cells recovered after thawing with reference to the initial number of fresh cells that were cryopreserved. In the case of the tumor samples (C) the cell count per mg of tissue was calculated from cells obtained after thawing and dissociation.

MACS Freezing Solution preserves the cellular composition of primary cell suspensions after thawing

The cellular composition of PBMCs and tumor cell suspensions was analyzed after thawing. Likewise, the cellular composition of frozen tumor samples was analyzed after thawing and dissociation. All cell populations detected in the fresh samples were present after cryopreservation with MACS Freezing Solution at similar frequencies. Comparable results were obtained with serum-containing medium (fig. 3).

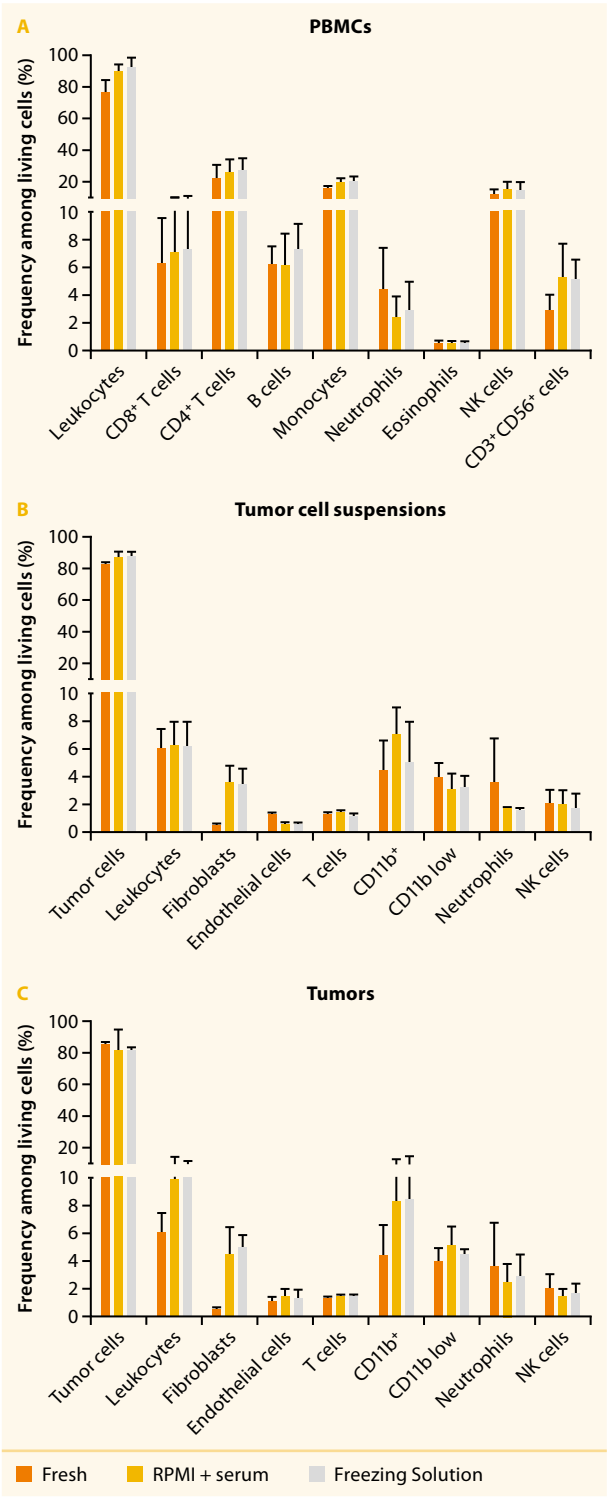

Figure 3: Analysis of the cellular composition of fresh and cryopreserved primary cells and tumors. PBMCs, tumor cell suspensions, and tumor samples were cryopreserved either using a serum-containing medium or the serum-free MACS Freezing Solution. The cellular composition of the fresh and thawed samples was analyzed. The cell frequencies are shown as the percentage among the total viable cells present in the samples.

Conclusions

- MACS Freezing Solution represents a ready-to-use and reliable serum-free cryopreservation medium for the long-term storage of primary cells and solid tissues, like PBMCs and tumors.
- Cell viability of primary cells cryopreserved using MACS Freezing Solution is well preserved after thawing, allowing cell recoveries comparable to or better than those cryopreserved with a serum-containing cryopreservation medium.
- MACS Freezing Solution also ensures the preservation of the cellular composition of the samples after thawing.

Product	Order no.
MACS Freezing Solution	130-129-552
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
8-Color Immunophenotyping Kit, human	130-120-640

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