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Optimal short-term storage of immune cells using MACS® Cell Storage Solution

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Background

Occasionally, primary cells need to be transported to a different location to proceed with downstream assays, or short-term storage may be required to continue the experiment in the following days. In such cases, it is crucial to provide the cells with an environment that does not induce apoptosis or stress, as this could interfere with downstream experiments. The MACS Cell Storage Solution is a ready-to-use, animal component- and serum-free medium specifically developed for short-term storage of primary cells, such as immune cells, from the blood.

In this study, we used the MACS Cell Storage Solution to store human lysed blood, peripheral blood mononuclear cells (PBMCs), $CD4^+$ T cells, and monocytes for up to 72 hours at 4 °C. We analyzed cell viability, recovery, and composition to evaluate the performance of our storage media. We also performed biological assays using stored $CD4^+$ T cells and monocytes to verify their functionality after storage. In addition, we compared the transcriptional signature of PBMCs which were stored in MACS Cell Storage Solution and another commercially available storage solution to investigate the impact of short-term storage on the transcriptional level.

Materials and methods

Storage of human lysed blood
Whole blood from three different donors was lysed using the Red Blood Cell Lysis Solution to remove erythrocytes. The resulting cell suspensions were aliquoted and stored in MACS Cell Storage Solution for 24, 48, or 72 hours at 4 °C at a concentration of 1×10^6 cells per mL of solution. Fresh cells were used as reference. Cell count, cell viability, and the cellular composition of all fresh and stored samples were analyzed by flow cytometry using the MACSQuant® Analyzer 10.

Cell count and cell viability were determined by volumetric pipetting and propidium iodide staining. The composition of the lysed blood cell suspensions was analyzed using the 8-Color Immunophenotyping Kit, human.

Monocyte-derived dendritic cell (Mo-DC) differentiation assay
The generation of Mo-DCs was performed as described in detail in our application note "Generation of Mo-DCs".[1] In brief, monocytes were isolated from PBMCs from three different donors using CD14 MicroBeads, human, and stored at a concentration of 1×10^6 cells per mL of MACS Cell Storage Solution for 72 hours at 4 °C. Fresh cells were used as reference. Fresh and stored monocytes were seeded at a density of 1×10^6 cells/mL in 3 mL per well for cultivation in 6-well plates in differentiation medium (RPMI-1640, 2 mM L-Glutamine, 1% human AB serum, 250 IU/ml IL-4 and 800 IU/ml GM-CSF). On day two, half of the medium was centrifuged at 300×g for 5 min at room temperature, the supernatant was discarded, and the cell pellet was resuspended in the same volume of fresh stimulation medium (RPMI-1640, 2 mM L-Glutamine, 1% human AB serum, 500 IU/ml IL-4, and 1600 IU/ml GM-CSF). Cells were differentiated into immature Mo-DCs until day six after the start of cultivation. For maturation of immature Mo-DCs, on day six half of the cultivation medium was centrifuged at 300×g for 5 min at room temperature, the supernatant was discarded, and the cell pellet was resuspended in the same volume of fresh maturation medium (RPMI-1640, 2 mM L-Glutamine, 1% human AB serum, 2000 IU/ml IL-6, 400 IU/ml IL-1β, 2000 IU/ml TNF-α, and 2 µg/ml PGE_2). Cells were matured for one additional day.

The phenotype of isolated monocytes and Mo-DCs was analyzed using REAfinity™ Recombinant Antibodies: CD1a, CD14, CD25, CD40, CD54, CD80, CD83, CD86, CD197, CD206, CD209, HLA-DR, and HLA-ABC. The phenotype of isolated naive T cells and proliferating T cells was analyzed using CD4, CD25, and CD69 REAfinity Antibodies. All flow cytometric analyses were performed using a MACSQuant Analyzer 10.[1]

T cell proliferation assay

Effector CD4+ T cells were isolated from PBMCs from two different donors by depleting CD25+ cells in the first place using CD25 MicroBeads II, human, and then enriching CD4+ T cells using the CD4+ T Cell Isolation Kit, human. Isolated T cells were stored at a concentration of 1×10^6 cells/mL of MACS Cell Storage Solution for 72 hours at 4 °C.

Fresh cells were used as reference. Fresh and stored cells were labeled using the CellTrace™ Violet Cell Proliferation Kit (Thermo Fisher Scientific®). To stimulate T cell proliferation, the T Cell Activation/Expansion Kit, human, was added to the cultivation medium (TexMACS™ Medium + 5% AB serum). The CellTrace Violet signal intensity was measured on days 0, 3, and 6 using a MACSQuant Analyzer 10.

Whole transcriptome analysis of PBMCs

The PBMCs from three different donors were split into two fractions for storage. One fraction was stored using MACS Cell Storage Solution and the other fraction using a different commercially available medium (Medium HT). Each PBMC fraction was aliquoted and stored for 24, 48, or 72 hours at 4 °C at a concentration of 1×10^6 cells/mL. Fresh cells were used as reference. RNA was isolated from fresh and stored cells using the RNeasy® Kit (QIAGEN®), following quantification of RNA using Qubit™ 4 Fluorometer (Thermo Fisher Scientific) and sample quality was determined with an automated electrophoresis tool using the 2100 Bioanalyzer™ Instrument (Agilent Technologies). The following library preparation was performed using QIAseq® Stranded mRNA Kit (QIAGEN). The library was sequenced on the NextSeq® 550 System (Illumina®). Transcriptome sequencing data analysis was performed via CLC Genomics Workbench/Server version 22.0 (QIAGEN) covering trimming of reads, read mapping (hg38, forward strand only), differential gene expression analysis ('While controlling for = Donor'), and principal component analysis. Genes with a false discovery rate of <0.05 and absolute fold changes >1.5 were considered to be differentially expressed.

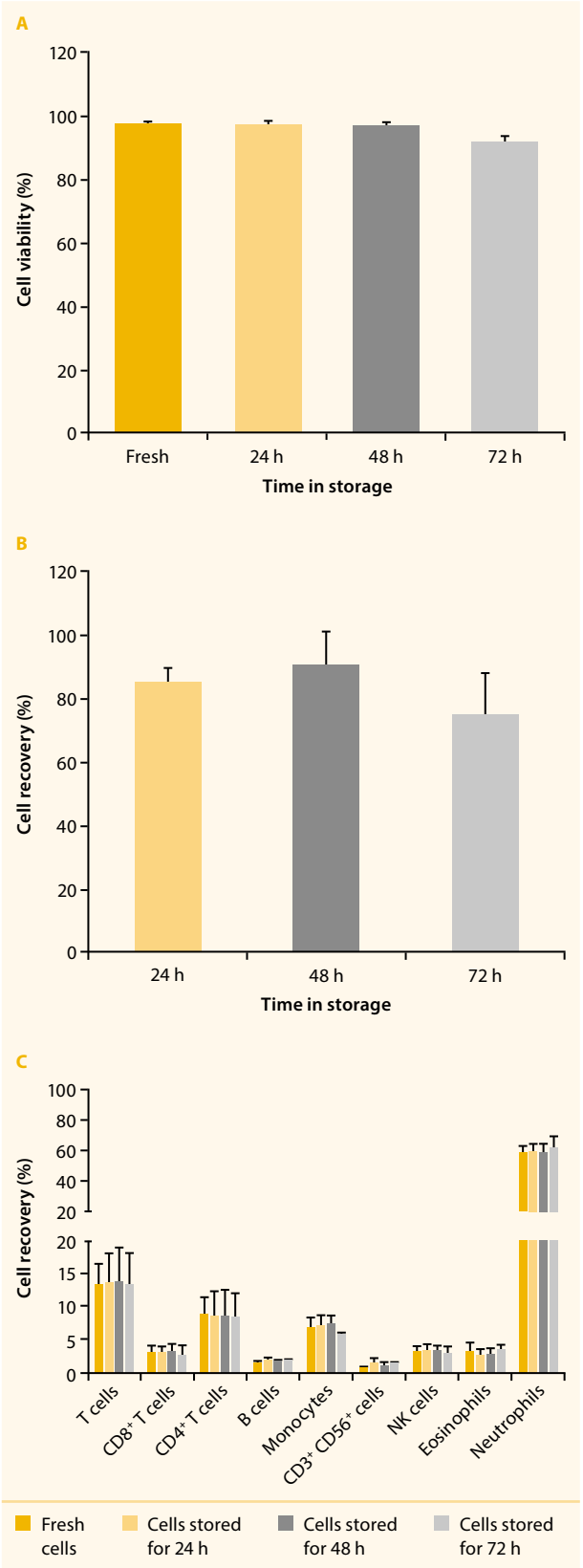

Figure 1: Analysis of immune cells from lysed blood after different storage times. Immune cells from whole blood were stored in MACS Cell Storage Solution at 4 °C for 24, 48, or 72 hours. The viability of fresh and stored cells was analyzed (A), and cell recoveries were determined for each storage time point as the percentage of viable cells recovered from the storage solution with respect to the initial number of cells that were stored (B). The frequencies of different immune cell phenotypes were analyzed for fresh and stored cells (C).

Results

MACS Cell Storage Solution preserves the viability and composition of immune cells from blood for up to 72 hours in storage

Immune cells from lysed blood were stored in MACS Cell Storage Solution for 24, 48, and 72 hours at 4 °C. Cell viability and composition were analyzed at each storage time point. Fresh samples were used as reference. The viability of stored cells was as high as the respective fresh cells for each analysis time point (fig. 1A). The recovery of viable cells was also above 80%, up to 48 hours of storage (fig. 1B). In addition, analysis of immune cell phenotypes from blood showed high concordance between fresh and all stored samples (fig. 1C).

T cells and monocytes retain their proliferation and differentiation capacities after being stored for 72 hours in MACS Cell Storage Solution

CD4⁺ T cells and monocytes were isolated from PBMCs and used for functional assays either fresh or after storage in MACS Cell Storage Solution at 4 °C for 72 hours. The viability of the stored cells was as high as for their respective control (fig. 2A), and cell recoveries were above 90% for both cell populations (fig. 2B).

The proliferation capacity of stored CD4⁺ T cells was assessed by measuring the dilution of a cell trace dye over a period of six days. Stored CD4⁺ T cells maintained proliferation capacity similar to fresh CD4⁺ T cells in culture (fig. 3A). After six days in culture, similar frequencies of fresh and stored CD4⁺ T cells expressed the activation markers CD25 and CD69 (fig. 3B).

In addition, the ability of stored monocytes to differentiate into Mo-DCs was determined using an established Mo-DC differentiation assay.[1] The stored monocytes retained their ability to differentiate into immature Mo-DCs after 72 hours of storage, although the number of differentiated cells was lower compared to the number of cells that had differentiated from fresh monocytes (data not shown). At day 7, cells that had differentiated into mature Mo-DCs (mMo-DCs) expressed characteristic dendritic cell markers at comparable levels, regardless of whether they had differentiated from fresh or stored monocytes (fig. 4).

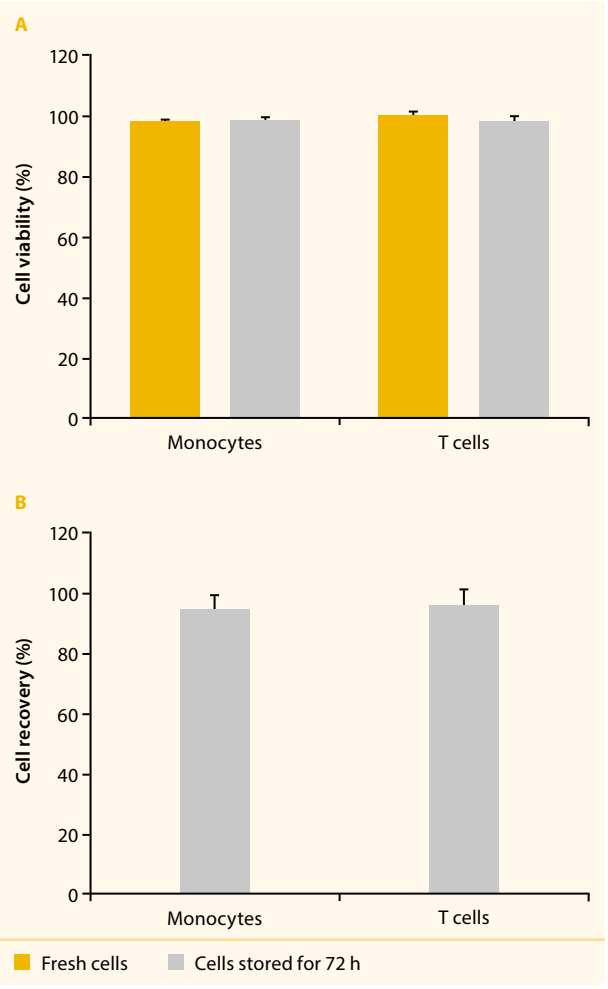

Figure 2: Cell viability and recovery of naive T cells and monocytes after 72 hours of storage. Naive T cells and monocytes were isolated from PBMCs and stored separately in MACS Cell Storage Solution for 72 hours. The viability (A) of fresh and stored cells and the recovery of viable cells after 72 hours of storage (B) were analyzed by flow cytometry.

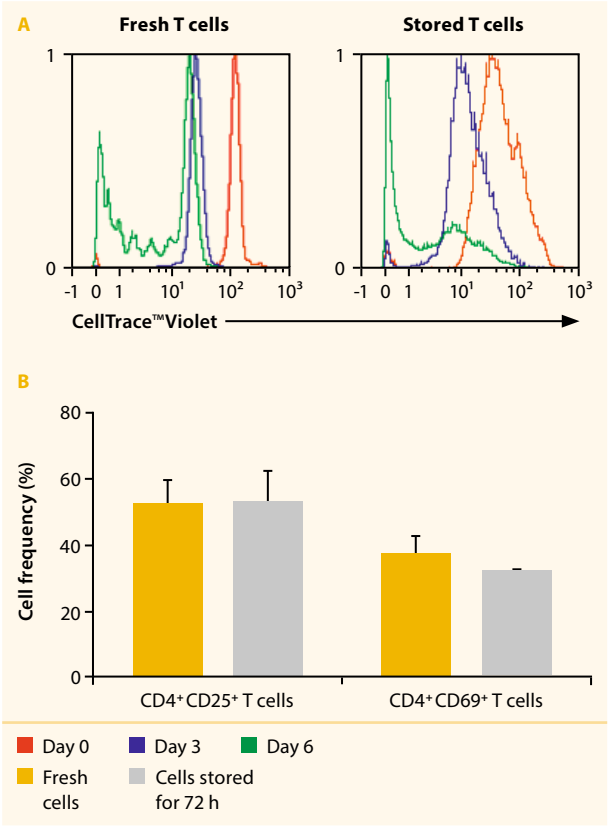

Figure 3: Analysis of the proliferation potential and phenotype of fresh and stored CD4⁺ T cells. CD4⁺ T cells, either fresh or stored for 72 hours in MACS Cell Storage Solution, were cultured for six days and their proliferation capacity and phenotype were evaluated. Prior to cultivation, cells were labeled with a cell trace dye to track the cell division, which was analyzed at day 3 and day 6 of culture (A). Expression of the activation markers CD25 and CD69 was analyzed on day 6 of culture (B).

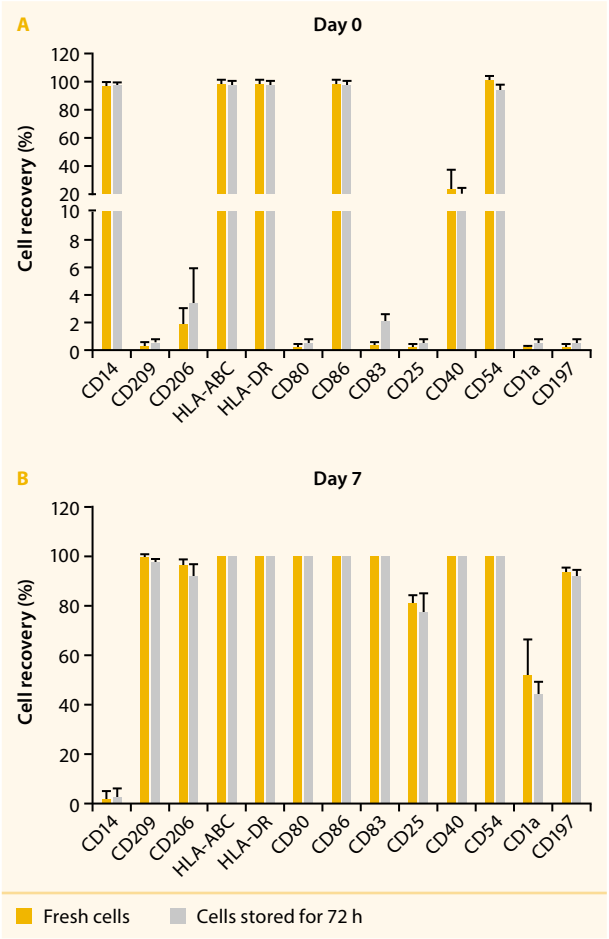

Figure 4: Phenotype of fresh and stored monocytes before and after differentiation to mature Mo-DCs. Monocytes either fresh or stored for 72 hours in MACS Cell Storage Solution were differentiated into Mo-DCs. The phenotype of the cells was analyzed on day 0 (monocytes) before starting the culture (A) and on day 7 (mMo-DCs) of differentiation (B).

Minimal cellular stress and toxicity responses of PBMCs stored in MACS Cell Storage Solution

The transcriptional signature of fresh and stored PBMCs from three donors was analyzed by whole transcriptome sequencing. One fraction of PBMCs was stored in MACS Cell Storage Solution and the second fraction of each corresponding donor was stored in Medium HT.

The heat map in figure 5 displays the number of differentially expressed genes detected for the different time points, showing an increase in number and magnitude with increasing storage time indicated in red. At all time points measured, a much higher number of genes was differentially expressed by cells stored in Medium HT compared to cells stored in MACS Cell Storage Solution (fig. 5). In addition to the mere number of differentially expressed genes, the fold changes in gene expression were also higher in cells stored in Medium HT, especially when the storage time exceeded 48 hours.

A closer look at 64 genes related to cellular stress and toxicity shows a higher effect of Medium HT, compared to MACS Cell Storage Solution (fig. 6). Among these 64 genes, GADD45G and Hmox1 are the most up- and down-regulated genes, respectively. Both indicate a higher stress level in cells stored in Medium HT.[2,3]

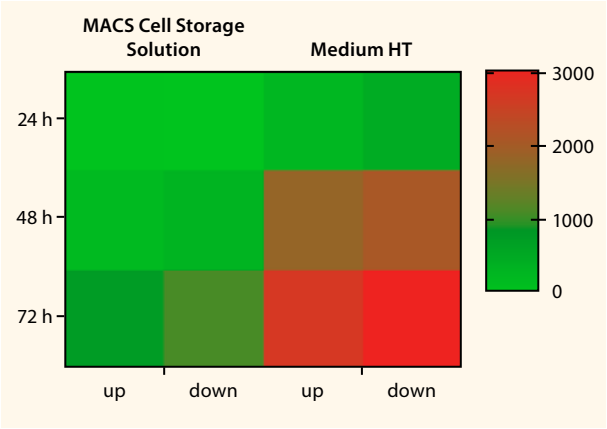

Figure 5: Heat map showing differentially expressed genes after whole transcriptome sequencing at different storage times and comparison between different storage solutions. Red color indicates a higher number of differentially expressed genes, while green color indicates no or only a minor number of differentially expressed genes.

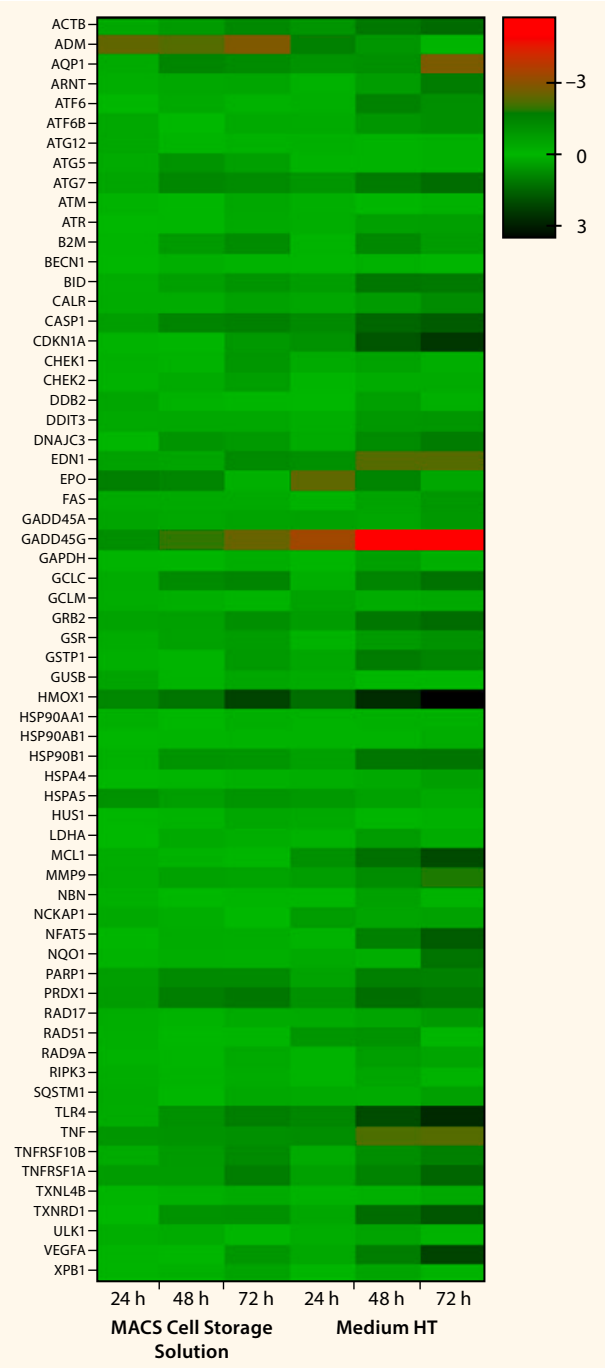

Figure 6: Heat map displaying log2 gene expression changes of 64 stress- and toxicity-associated genes. Red color indicates up-regulation and black color indicates down-regulation of the respective gene under the examined conditions, whereas green color indicates no or only minor changes in gene regulation.

Conclusions

In this application note we analyzed short-term storage of immune cells using our MACS Cell Storage Solution. We showed that:

- MACS Cell Storage Solution preserves the viability and cellular composition of immune cell suspensions for up to 72 hours.
- Stored cells retain their functional proliferation and differentiation capacities after 72 hours of storage.
- Cell stress responses during short-term storage were efficiently minimized when using MACS Cell Storage Solution.

Product	Order no.
MACS Cell Storage Solution	130-130-263
TexMACS Medium	130-097-196
Red Blood Cell Lysis Solution (10×)	130-094-183
CD14 MicroBeads, human	130-050-201
CD25 MicroBeads II, human	130-092-983
CD4+ T Cell Isolation Kit, human	130-096-533
T Cell Activation/Expansion Kit, human	130-091-441
MACSQuant Analyzer 10	130-096-343
8-Color Immunophenotyping Kit, anti-human	130-120-640
Mo-DC Analysis Cocktail Kit, anti-human	130-093-567
Human GM-CSF, research grade (10 µg)	130-093-862*
Human IL-4, research grade (10 µg)	130-095-373*
Human TNF-α, research grade (10 µg)	130-094-015*
REAfinity Recombinant Antibodies	**

* Order numbers are provided for 10 µg sizes. For different quality grades and additional package sizes, visit **www.miltenyibiotec.com/cytokines**

** For more information on REAfinity Recombinant Antibodies conjugated to a wide range of fluorochromes visit **www.miltenyibiotec.com/antibodies**

LEARN MORE

Find more information on automated preparation of PBMCs directly from blood with MACS Technology here:
▸ **miltenyibiotec.com/pbmc-isolation-kit**

References

1. Generation of Mo-DCs - From monocyte isolation to DC characterization (2016). Application note, Miltenyi Biotec. miltenyibiotec.com/mo-dc_AppNote
2. Takekawa, M. and Saito, H. (1998) A family of stress-inducible GADD45-like proteins mediate activation of the stress-responsive MTK1/MEKK4 MAPKKK. Cell 95: P521–530.
3. Poss, K.D. and Tonegawa, S. (1997) Reduced stress defense in heme oxygenase 1-deficient cells. Proc. Natl. Acad. Sci. USA 94: 10925–10930.

Notes

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