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Stability of Supplemented PRIME-XV T Cell CDM at Room Temperature

TECHNICAL NOTE

INTRODUCTION

To preserve the functionality and performance of PRIME-XV T Cell CDM, and to maintain the stability of the medium when supplemented, it is stored in a temperature controlled environment and protected from light until ready to use. Although it is shipped at 2–8°C on cold packs, there can be delays by the carrier or other unforeseen challenges that lead to the temperature not being maintained. Also, during preparation for use in cell culture, the supplemented medium may be kept at room temperature for extended periods of time. Furthermore, when cytokines are added to the medium by the end-user to prepare it for use in cell culture, these can also degrade over time. End-users need reassurance that the functionality of the medium will be maintained throughout the cell culture process, even if there is a temperature excursion or the medium is kept at room temperature for some time.

We have investigated the performance stability of supplemented PRIME-XV T Cell CDM kept at room temperature for various durations, from 6 hours to 72 hours. Performance of the medium was evaluated in cell culture, as indicated by cell count and viability, as well as marker expression. Human peripheral blood mononuclear cells (PBMCs) from two donors were used to perform two separate, but similar, experimental runs (Run 1 and Run 2). The results presented here confirm that while there is donor-to-donor variability, and the cells have different growth curves, the supplemented PRIME-XV T Cell CDM continues to perform as intended even when kept at room temperature for up to 72 hours.

PRIME-XV T Cell CDM is a chemically defined, animal component-free medium optimized for the growth and expansion of human T cells. The medium contains components that are essential for maintaining optimal growth under physiological conditions including carbohydrates, amino acids, vitamins, and minerals, some of which are temperature sensitive.

MATERIALS

- PRIME-XV T Cell CDM (Catalog # 91154), FUJIFILM Irvine Scientific, Santa Ana, CA
- CTGrade GMP rh IL-2_{C126S} (Catalog # 500-01), CTGrade GMP rh IL-7 (Catalog # 500-07), CTGrade GMP rh IL-15 (Catalog # 500-08), and CTGrade GMP rh IL-21 (Catalog # 500-09), FUJIFILM Irvine Scientific, Warminster, PA
- Anti-human CD3 Antibody (Catalog # 317347) and Anti-human CD28 Antibody (Catalog # 302959), BioLegend, San Diego, CA
- PBMC (Catalog # PB009C2), Charles River Laboratories, San Diego, CA
- G-Rex 24-Well Plate (Catalog # 80192M), Wilson Wolf Corporation, St Paul, MN
- APC/Cyanine7 Anti-Human CD3 Antibody (Catalog # 344818), BioLegend, San Diego, CA
- Alexa Fluor 700 anti-human CD8 Antibody (Catalog # 344724), BioLegend, San Diego, CA
- BD Horizon BUV496 Mouse Anti-Human CD4 Antibody (Catalog # 612936), BD Bioscience, San Diego, CA
- LIVE/DEAD Fixable Blue Dead Cell Stain Kit (Catalog # L34962), ThermoFisher Scientific, Carlsbad, CA

METHOD

Sample Preparation

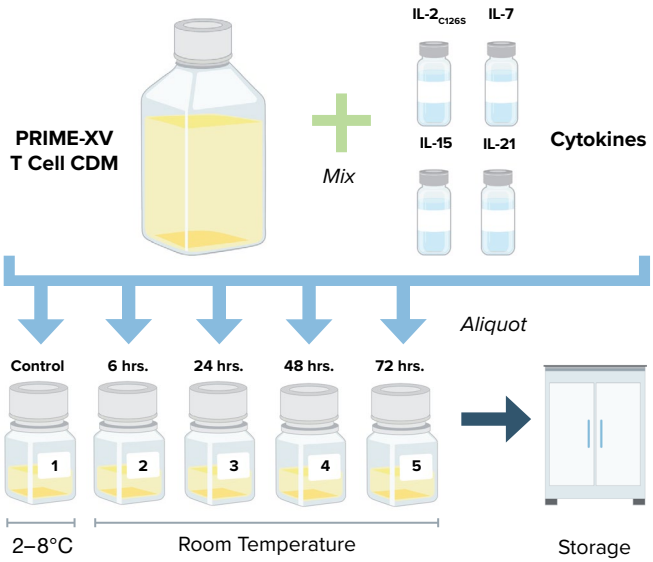
PRIME-XV T Cell CDM was supplemented with standard concentrations of IL-2_{C126S}, IL-7, IL-15, and IL-21. The supplemented medium was aliquotted into 40 mL samples. The test samples were stored at room temperature in a lightproof cabinet for 6, 24, 48, or 72 hours, and the control sample was stored at 2–8°C. All samples were brought to working temperature just before use. **(See Figure 1, Step 1)**

Cell Expansion

PBMCs were thawed and seeded in a T-75 flask and allowed to recover overnight. In addition to the cytokines, anti-CD3 and anti-CD28 antibodies were added to the control and test medium samples. The cells were stimulated with the medium samples containing the mixture of cytokines and antibodies for 24 hours. The following day, the cells were seeded with the supplemented medium samples at 1 x 10^6 cells per well (5 x 10^5 cells/cm^2) in a G-Rex 24-Well Plate. After 7 days of expansion, upon reaching confluence, the cells were transferred to T-75 flasks. The culture ended on Day 10. **(See Figure 1, Step 2 and 3)** Cells were maintained at 37°C and 5% CO_2 in a humidified incubator. PBMCs from two different donors were assessed in this manner, and are presented as Run 1 and Run 2.

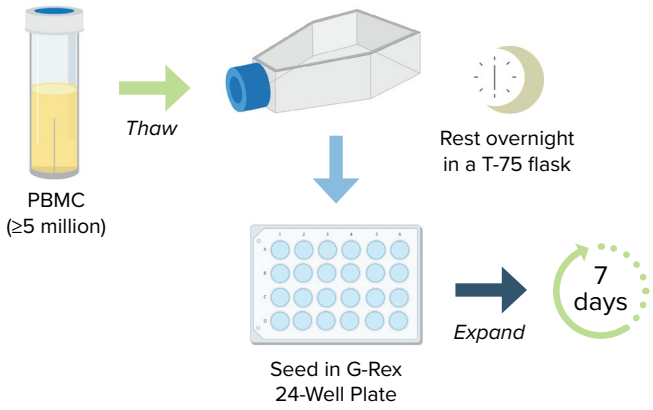
Cell Count and Flow Cytometry Analysis

The expanded PBMCs were washed by adding Phosphate-Buffered Saline (PBS) (without Ca^{+2} and Mg^{+2}) and centrifugation at 300 xg for 5 minutes. The cells were resuspended in PBS, and aliquotted for counting and viability. 100 µL PBS was mixed with 1 µL LIVE/DEAD Fixable Blue Dead Cell Stain and added to each well or tube containing the cells, and then incubated at 4°C for 30 minutes. To prepare for flow cytometry, the cells were washed in flow buffer and stained in Brilliant Stain Buffer Plus with a 1/500 dilution of fluorescent antibodies against CD3, CD4, and CD8. The cells were incubated for 30 minutes at 4°C, washed with flow buffer twice, and then resuspended in 300 µL of flow buffer. The samples were analyzed using a cell analyzer to measure expression markers. **(See Figure 1, Step 3)**



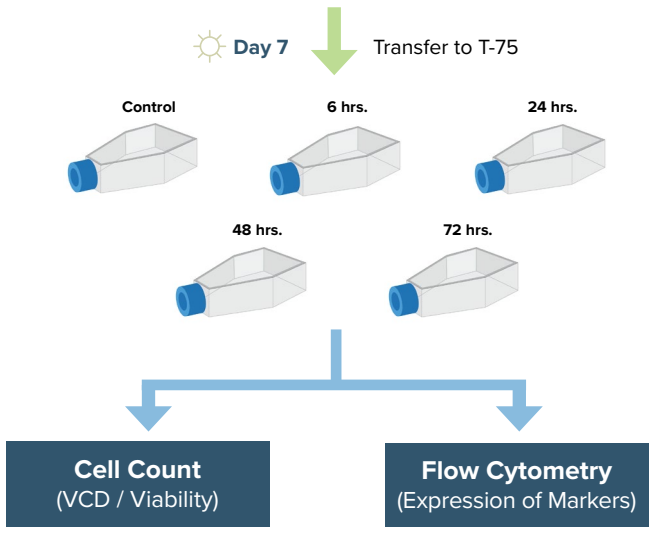
STEP 1

- Add Cytokines to PRIME-XV T Cell CDM
- Prepare aliquots for test conditions
- Store as indicated



STEP 2

- Thaw cells and rest overnight in T-75
- Seed cells in G-Rex 24-Well Plate
- Expand cells for 7 days



STEP 3

- Transfer cells to T-75 flask on Day 7
- Expand cells through Day 10
- Harvest cells and Analyze

Figure 1. A schematic illustration of the experimental set-up.

RESULTS

Supplemented PRIME-XV T Cell CDM Performs as Intended Even When Stored at Room Temperature for Up to 72 Hours

The result for the cell expansion runs using supplemented media kept at room temperature for various durations is shown below. Cell counts and percent viability after expansion for 3, 7, and 10 days in Run 1 and Run 2 are shown in **Figure 2**.

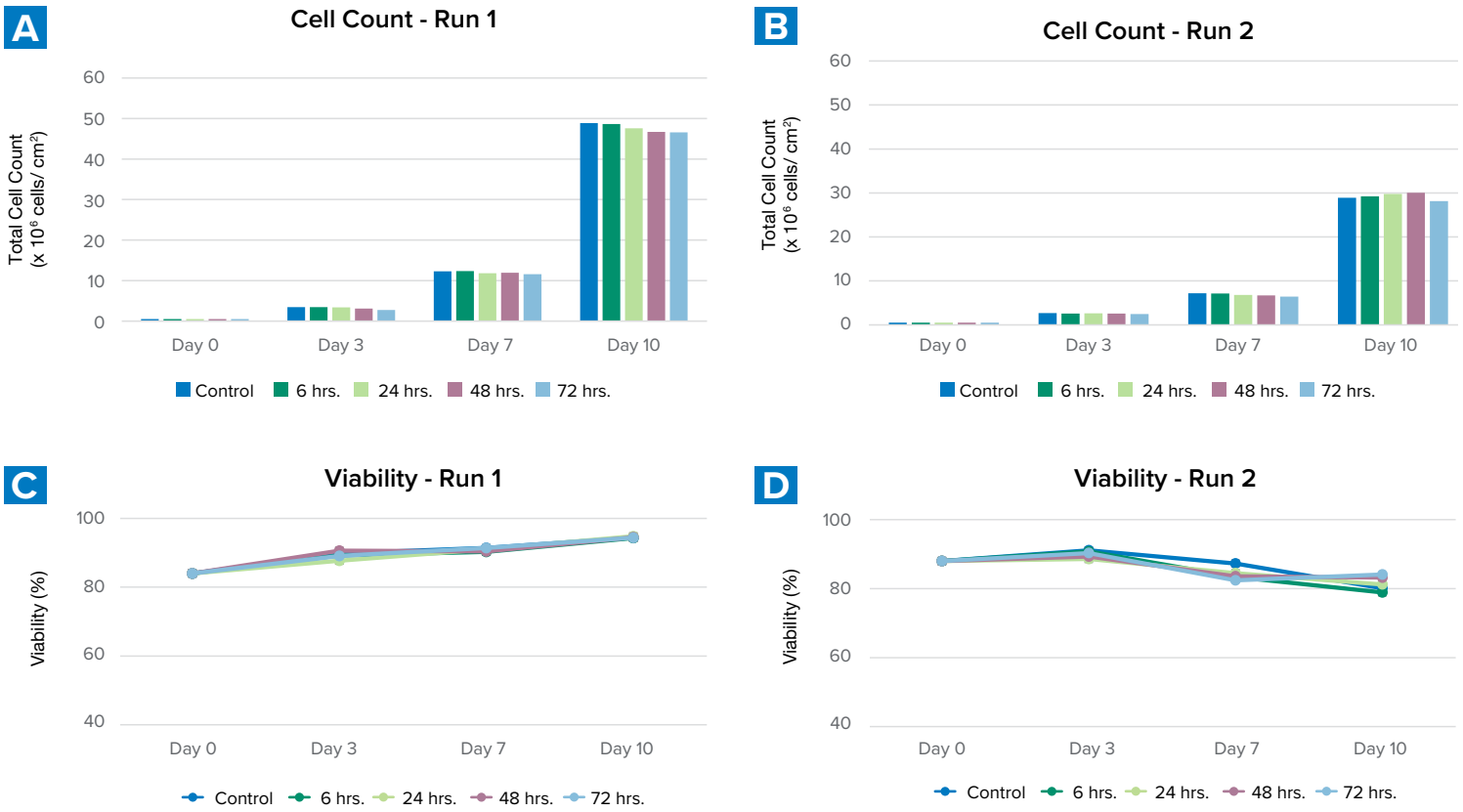


Figure 2. Cell expansion results for Run 1 and Run 2. The total cell counts for **(A)** Run 1, **(B)** Run 2, and the viability (%) for **(C)** Run 1, and **(D)** Run 2 using supplemented medium stored for various durations at room temperature.

Supplemented PRIME-XV T Cell CDM Stored at Room Temperature Supports Robust Fold Expansion of PBMCs

Starting with 1 x 10^6 cells, the fold expansion of the cells for Days 3, 7, and 10 for Run 1 and Run 2 was calculated, and is shown in **Table 1**.

Table 1. Fold Expansion of PBMCs

	Run 1			Run 2		
	Day 3	Day 7	Day 10	Day 3	Day 7	Day 10
Control	6.9	24.6	97.8	5.4	14.4	57.8
6h	7.0	24.7	97.3	5.2	14.3	58.5
24h	6.8	23.7	95.2	5.3	13.7	59.6
48h	6.2	23.9	93.4	5.2	13.4	60.1
72h	5.5	23.2	93.1	5.0	12.9	56.3

Media Stored at Room Temperature Does Not Negatively Affect Day 10 Surface Marker Expression of PBMCs

Surface marker expression for cells cultured for 10 days using supplemented media kept at room temperature for various durations for Run 1 and Run 2 was analyzed. The data was obtained using flow cytometry analysis to quantify the frequency and distribution of T cell subsets by gating specific cell populations according to their CD3, CD4, and CD8 expression. The results for the marker expression analysis are shown below. Percent of CD3-positive cells in the two runs are shown in **Figures 3A and 3B** and the CD4 and CD8 expression in the CD3-positive cells in the two runs is shown in **Figures 3C and 3D**.

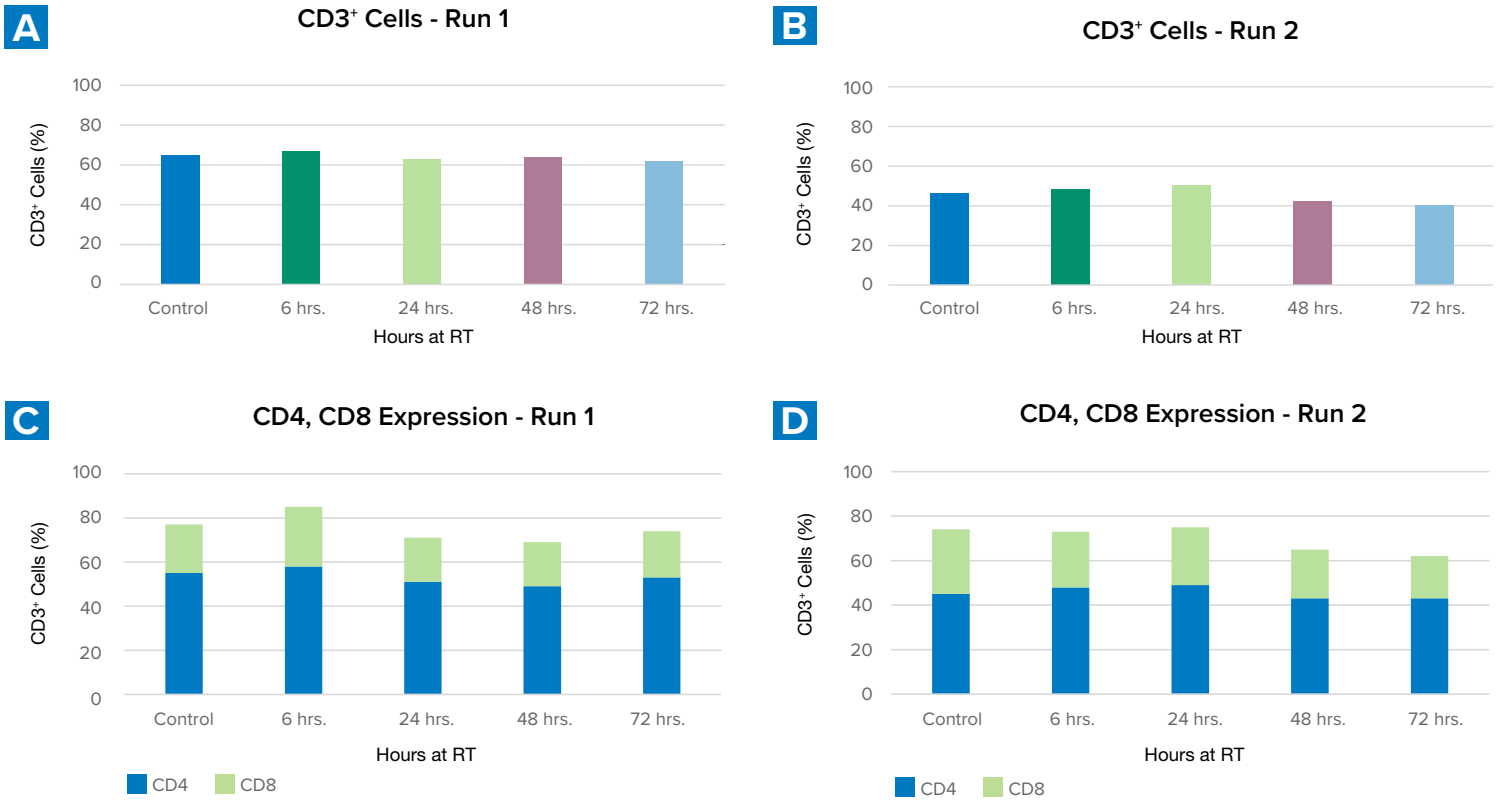


Figure 3. Flow cytometry analysis for cell surface marker expression. CD3⁺ cells from **(A)** Run 1, **(B)** Run 2; and CD4 and CD8 expression levels in the CD3⁺ cells from **(C)** Run 1 and **(D)** Run 2.

DISCUSSION

Cells grown in cytokine-supplemented PRIME-XV T Cell CDM that had been kept at room temperature before use showed similar cell count and viability profiles on Days 3, 7, and 10 for both runs, demonstrating that the medium remains stable for up to 72 hours at room temperature. In addition to measuring final cell count and viability, a flow cytometry assay was conducted to analyze the marker expression on the PBMCs to evaluate the expanded cells qualitatively. Because PBMCs were sourced from two different donors, a difference in total cell numbers between the two runs was observed. However, within each run, the difference between media samples stored for various durations at room temperature were negligible. The percentage of CD3⁺ cells obtained at the end of the culture run was also comparable for the different conditions within each run. Finally, the CD4/CD8 expression levels remained stable. Together, these results suggest that the cytokine-supplemented PRIME-XV T Cell CDM remains stable and supports T cell expansion as intended, even when exposed to room temperature for up to 72 hours.

CONCLUSION

This study examined the effect of room temperature storage on cytokine-supplemented PRIME-XV T Cell CDM and its ability to support T cell expansion. Results show that room temperature storage from 6 hours to 72 hours did not have any significant effect on its ability to support T cell expansion. In particular, room temperature storage of the medium did not affect cell proliferation, viability, or cell surface marker expression.

In conclusion, the storage of cytokine-supplemented PRIME-XV T Cell CDM at room temperature for up to 72 hours has no adverse effect on T cell activation, expansion, and cell surface phenotype. T cell expansion was similar to that for control medium. End-users can rest assured that cytokine-supplemented PRIME-XV T Cell CDM will continue to perform as intended even if stored at room temperature for up to 72 hours. The recommendation, however, is to store the medium at 2–8°C and protect from light until ready to use.

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