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Expanding PBMCs with PRIME-XV T Cell CDM and Shenandoah CTGrade GMP Recombinant Human Proteins in the G-Rex Cell Culture System

Application note

SUMMARY

This application note provides data on the activation and expansion of peripheral blood mononuclear cells (PBMCs) from three different donors in PRIME-XV T Cell CDM using Shenandoah CTGrade GMP rh IL-2_{C126S}, IL-7, IL-15, and IL-21 (Catalog # 500-01, 500-07, 500-08, and 500-09, respectively) in a 24-well G-Rex cell culture system.

INTRODUCTION

The γ_c family of cytokines is known for the complementary, overlapping, and unique immunomodulatory functions among associated interleukins. As a member of the γ_c family, interleukin 2 (IL-2) is a vital regulator of the human immune response. Produced and consumed primarily by T cells, IL-2 stimulates the expansion and activity of adaptive and innate immune cells. While IL-2 is the most widely used γ_c cytokine for T cell expansion, other members of the family have also been well documented to impact the expansion and development of T cells in nuanced ways. Interleukins, such as IL-7, IL-15, and IL-21, can affect the distributions of memory cell populations, tolerance, polarization, and various aspects of the adaptive and innate immune response, and they are quickly gaining relevance in the development of IL-2-independent cell therapy products. The γ_c family interleukins are critical components for the production of many immunotherapies and cell therapies, requiring high-quality recombinant proteins for their manufacture. More specifically, the manufacture of therapeutics with reliable recombinant proteins minimizes variability and facilitates a smooth transition from early phases of product development to clinical and commercial applications.

CTGrade GMP recombinant human proteins from FUJIFILM Irvine Scientific are produced in an *E. coli* system and provide consistent biological activity and reproducible performance from lot to lot. Manufactured using current good manufacturing practices (cGMP) in ISO 9001:2015 certified facilities, these CTGrade GMP cytokines are compatible with our Research Use Only (RUO) recombinant proteins, ensuring minimal variability when transitioning from preclinical development to cGMP manufacturing of therapies and other products.

MATERIALS

Reagents

- PRIME-XV T Cell CDM
  (FUJIFILM Irvine Scientific, Catalog # 91154)
- Phosphate Buffered Saline (PBS)
  (FUJIFILM Irvine Scientific, Catalog # 9240)
- Dynabeads
  (ThermoFisher, Catalog # 11132D)
- CTGrade GMP rh IL-2_{C126S}
  (FUJIFILM Irvine Scientific, Catalog # 500-01)
- CTGrade GMP rh IL-7
  (FUJIFILM Irvine Scientific, Catalog # 500-07)
- CTGrade GMP rh IL-15
  (FUJIFILM Irvine Scientific, Catalog # 500-08)
- CTGrade GMP rh IL-21
  (FUJIFILM Irvine Scientific, Catalog # 500-09)
- Fresh human PBMCs
  (StemExpress, Catalog # PBMNC300F)

Disposables

- Gas Permeable Rapid Expansion (G-Rex) cell culture system
  (WilsonWolf Manufacturing # 80192M)
- 15 mL sterile conical tubes
- P1000, P200, P20, and P2 micropipette sterile tips
- 5, 10, and 25 mL serological pipettes

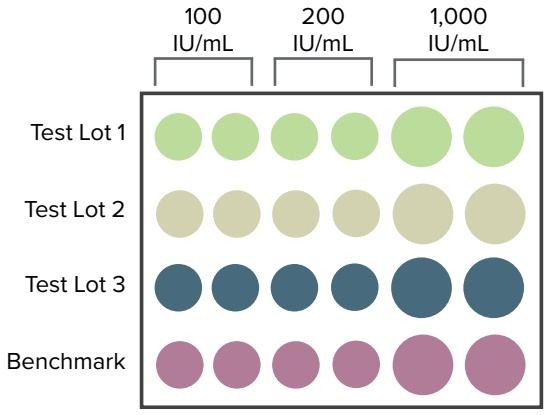
Equipment

- P1000, P200, P20, and P2 micropipettes
- Serological pipette controller
- Centrifuge
- Humidified tissue culture incubator
- ViCell XR cell analyzer
- FACSymphony A3

Method 1: CTGrade GMP rh IL-2_{C126S} with PRIME-XV T Cell CDM

Experimental design

- Expand in 24-well G-Rex cell culture vessels
- Test with freshly isolated PBMCs from 3 separate donors
- Culture each condition in duplicate
- Individual plates for each of 4 time points (days 3, 7, 10, and 13)
- 75% media change for relevant plates on days 5 and 10



Media preparation

1. Determine volume needed per media condition
   a. 12 separate conditions total (4 lots of CTGrade GMP rh IL-2_{C126S} at 3 concentrations)
      i. CTGrade GMP rh IL-2_{C126S}: 100 IU/mL, 200 IU/mL, and 1,000 IU/mL
   b. Each condition will have 2 wells of 7 mL culture volume per plate
   c. 3 plates per time point (for each of 3 donors)
   d. Day 3 plates will not need a media change
   e. Day 7 and day 10 plates will each have 1 media change (5.25 mL per well)
   f. Day 13 plates will need 2 media changes (11 mL per well)
   g. Total volume per condition needed:

      [1 plate x (2 wells x 7 mL/well) + 2 plates x (2 wells x (7 mL/well + 5.25 mL/well)) + 1 plate x (2 wells x (7 mL/well + 11.5 mL/well))] x 3 donors **= 330 mL per condition**

2. Prepare 350 mL PRIME-XV T Cell CDM stock with 3 concentrations of CTGrade GMP rh IL-2_{C126S} for each lot

   Extra volume for margin of error

Plate cells

3. Calculate number of cells needed per donor
   a. Seeding density = $5x10^5$ cells/cm^2
   b. 24 wells per plate
   c. 4 time points (plates) per donor

      $5x10^5$ cells/cm^2 x 2 cm^2/well x 24 wells/plate x 4 plates/donor = **$9.6x10^7$ cells/donor**

4. Activate $9.6x10^7$ cells per donor with Dynabeads per the protocol

   $9.6x10^7$ cells x 1 Dynabead/cell x 1 mL/$4x10^7$ Dynabeads
   = 2.4 mL Dynabeads per donor

5. Following activation with Dynabeads, resuspend each donor in 9.6 mL PRIME-XV T Cell CDM without CTGrade GMP rh IL-2_{C126S} and pipette well to create a homogenous cell suspension
6. Aliquot 100 µL of the cell suspension into each well of 4 individual 24-well G-Rex plates per donor
7. Add 7 mL appropriate media from **Step 2** into the corresponding wells of each plate and incubate in standard tissue culture conditions

Harvest and analyze cells

8. Feed and harvest plates at appropriate time points
    a. Harvest the day 3 plates, count the cells, and stain with the PBMC flow cytometry panel
    b. Feed day 7, 10, and 13 plates on day 5 by aspirating 5.25 mL of media from each well and replacing it with 5.25 mL of fresh, CTGrade GMP rh IL-2_{C126S} -supplemented media
    c. Harvest the day 7 plates, count the cells, and stain with the PBMC flow cytometry panel
    d. Feed day 13 plates on day 5 by aspirating 5.25 mL of media from each well and replacing it with 5.25 mL of fresh, CTGrade GMP rh IL-2_{C126S} -supplemented media
9. On the last day of the experiment, harvest day 13 plates and split the cell population into 3 groups: cells for counting, cells for surface staining, and cells for function testing and intracellular staining
    a. Count cells and stain for surface markers as with previous time points
10. Prepare the function test
    a. Plate approximately $2x10^6$ cells/well into duplicate 96-well round-bottom lidded plates (one will be the unstimulated control)
    b. Stain both plates for CD107a
    c. Stimulate 1 plate with staphylococcal enterotoxin B (SEB)
    d. Incubate both plates for 2 hours in standard tissue culture conditions
    e. Add protein transport inhibitor to both plates and incubate for 4 hours
11. Initiate intracellular staining
    a. Fix and permeabilize the cells
    b. Stain cells for intracellular markers and cytokines
    c. Run flow cytometry

RESULTS

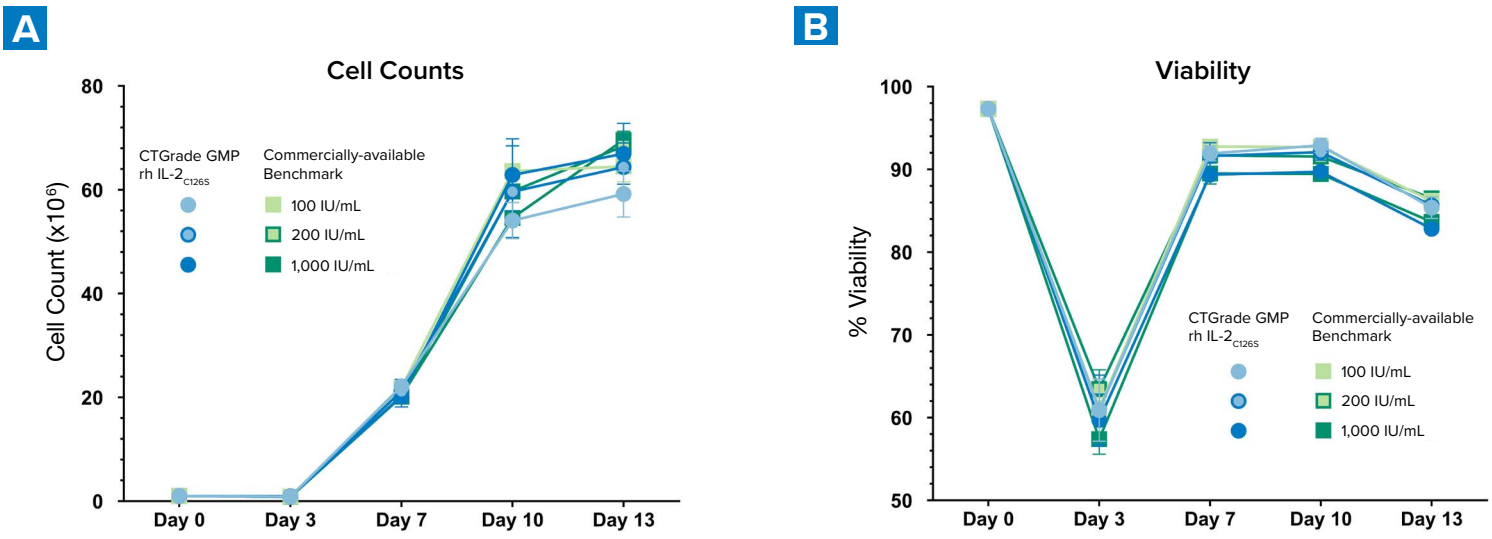


Figure 1. Fresh human PBMCs show robust expansion and high viability in PRIME-XV T Cell CDM supplemented with different concentrations of CTGrade GMP rh IL-2_{C126S}. (A) By Day 13, the cells cultured at the 2 higher concentrations of CTGrade GMP rh IL-2_{C126S} showed no significant difference in cell growth, while cells in the lowest CTGrade GMP rh IL-2_{C126S} concentration did not expand as well. This implies a functional saturation of CTGrade GMP rh IL-2_{C126S} is around 200 IU/mL. **(B)** The Day 3 drop in viability is commonly seen due to activation-induced apoptosis, and it is followed by complete recovery on Day 7. Cell viability remains above 85% over the course of the culture, and it is equally supported by all concentrations and sources of CTGrade GMP rh IL-2_{C126S}. Results are representative of 3 donors and 3 lots of CTGrade GMP rh IL-2_{C126S} run in duplicates per condition.

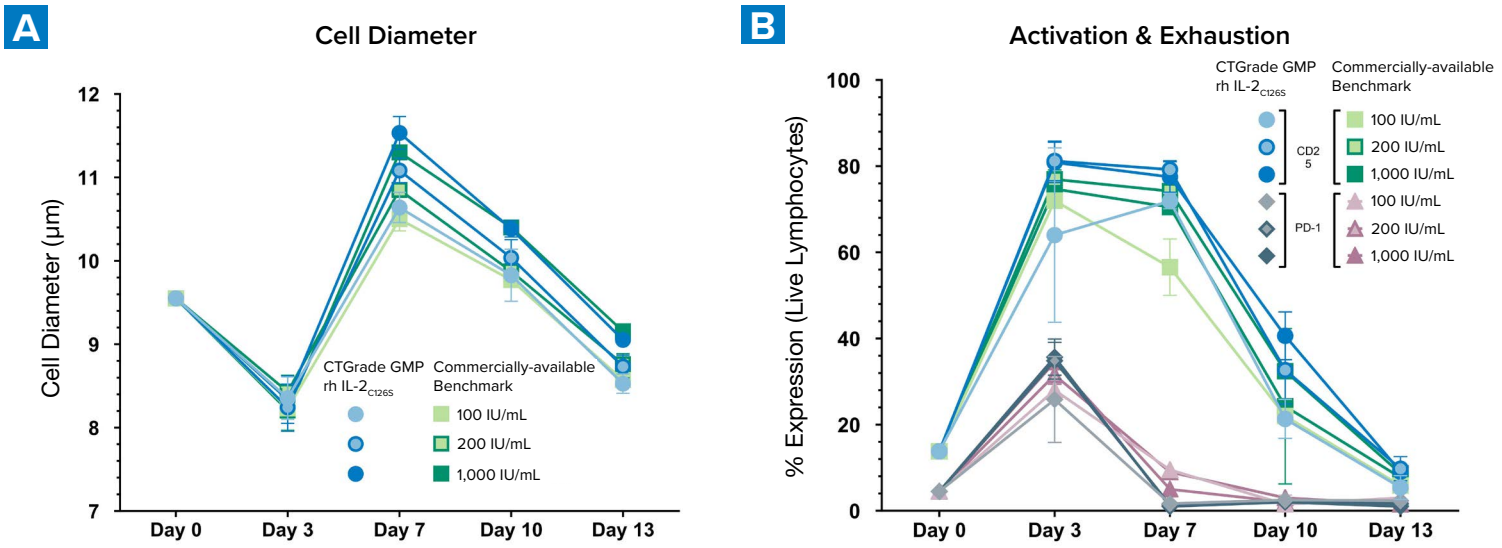


Figure 2. Fresh human PBMCs show healthy activation kinetics in PRIME-XV T Cell CDM supplemented with different concentrations of CTGrade GMP rh IL-2_{C126S}. (A) The increase in cell diameter post-activation is mildly dose-dependent. **(B)** The Day 3 drop in viability is accompanied by an increase in PD-1, which decreases to negligible levels by Day 7 post-expansion. The activation marker CD25 remains high through the first week of expansion, with a mild decrease in levels at the lowest CTGrade GMP rh IL-2_{C126S} concentration, returning to baseline by Day 13. Results are representative of 3 donors and 3 lots of CTGrade GMP rh IL-2_{C126S} run in duplicates per condition.

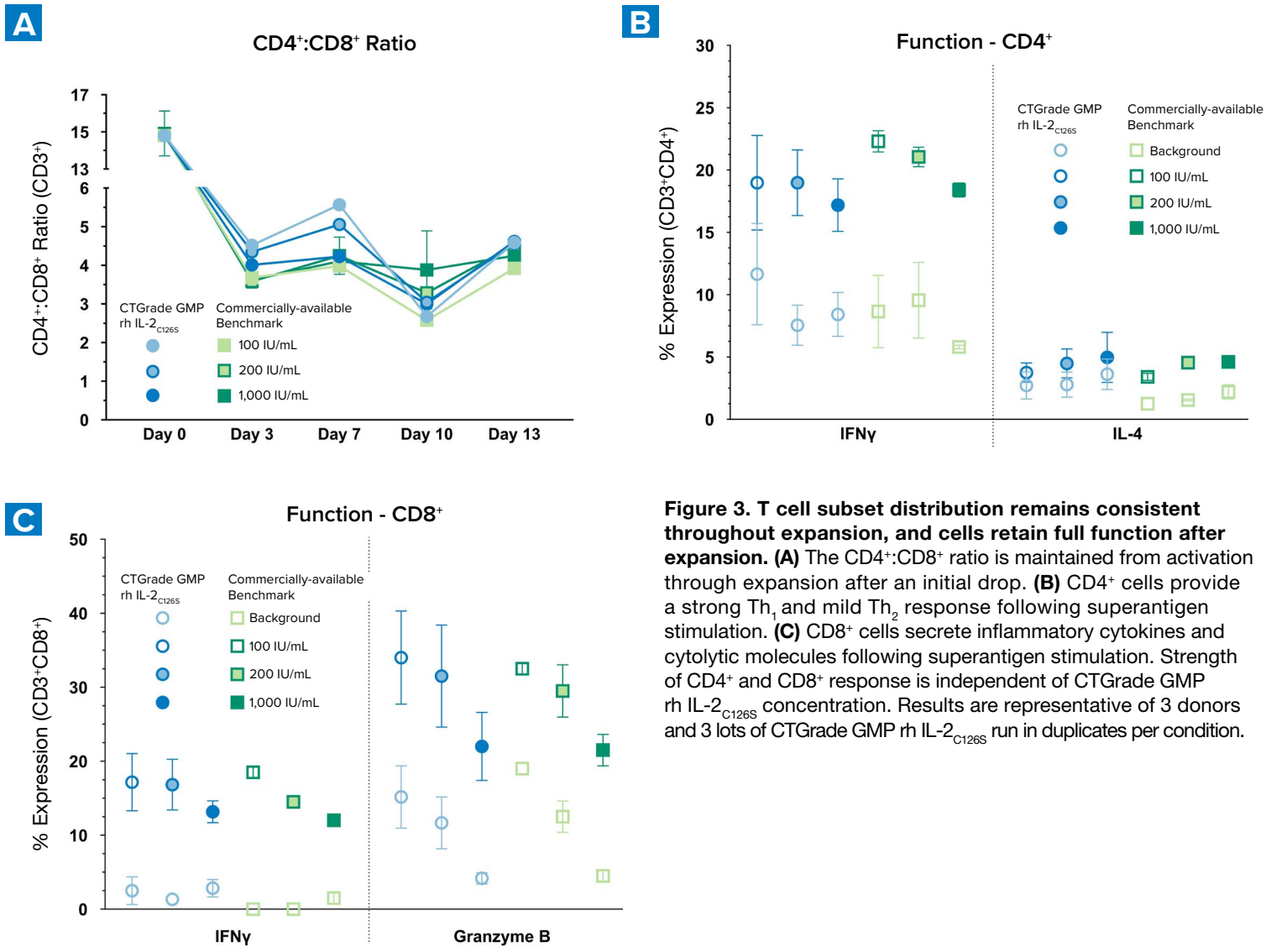
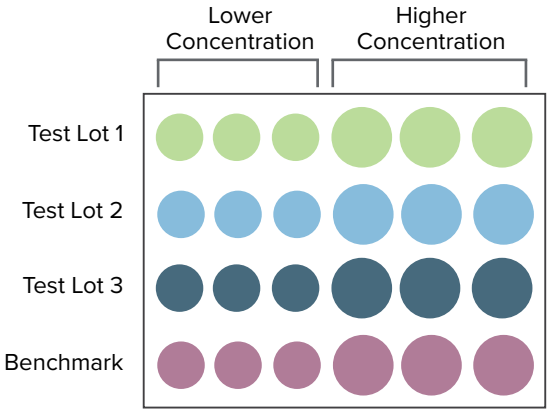


Figure 3. T cell subset distribution remains consistent throughout expansion, and cells retain full function after expansion. (A) The CD4+:CD8+ ratio is maintained from activation through expansion after an initial drop. **(B)** CD4+ cells provide a strong Th_1 and mild Th_2 response following superantigen stimulation. **(C)** CD8+ cells secrete inflammatory cytokines and cytolytic molecules following superantigen stimulation. Strength of CD4+ and CD8+ response is independent of CTGrade GMP rh IL-2_{C126S} concentration. Results are representative of 3 donors and 3 lots of CTGrade GMP rh IL-2_{C126S} run in duplicates per condition.

Method 2: CTGrade GMP rh IL-7, 15, 21 with PRIME-XV T Cell CDM

Experimental design

- Expand in 24-well G-Rex cell culture vessels
- Test with freshly isolated PBMCs from 3 separate donors
- Culture each condition in triplicate
- Individual plates for each of 3 time points (days 3, 7, and 10)
- 75% media change for relevant plates on days 5



Media preparation

1. Determine volume needed per media condition
   a. 24 separate conditions total (4 lots of each cytokine, 3 donors, 2 concentrations)
      i. CTGrade GMP rh IL-7: 5 ng/mL and 10 ng/mL
      ii. CTGrade GMP rh IL-15: 5 ng/mL and 10 ng/mL
      iii. CTGrade GMP rh IL-21: 10 ng/mL and 20 ng/mL
      iv. Add 200 IU/mL CTGrade GMP rh IL-2_{C126S} to IL-21 conditions since IL-21 is a complimentary cytokine that does not support good T cell expansion alone
   b. Each condition will have 3 wells of 7 mL culture volume per plate
   c. 9 plates per time point (for each of 3 donors and 3 cytokines)
   d. Day 3 plates will not need a media change
   e. Day 7 and 10 plates will each have 1 media change (5.25 mL per well)
   f. Total volume per condition needed:
      [3 plates x (3 wells x 7 mL/well) + 6 plates x (3 wells x (7 mL/well + 5.25 mL/well))] x 3 donors = **724 mL per condition**

2. Prepare 750 mL PRIME-XV T Cell CDM stock with 2 concentrations of each cytokine individually for each individual lot

   Extra volume for margin of error

Plate cells

3. Calculate number of cells needed per donor
   a. Seeding density = $5x10^5$ cells/cm^2
   b. 24 wells per plate
   c. 9 plates per donor (3 time points, 3 cytokines)
      $5x10^5$ cells/cm^2 x 2 cm^2/well x 24 wells/plate x 9 plates/donor = **$2.16x10^8$ cells/donor**
4. Activate $2.16x10^8$ cells per donor with Dynabeads per the protocol
   $2.16x10^8$ cells x 1 Dynabead/cell x 1 mL/$4x10^7$ Dynabeads
   = 5.4 mL Dynabeads per donor
5. Following activation with Dynabeads, resuspend each donor in 21.6 mL PRIME-XV T Cell CDM without cytokines and pipette well to create a homogenous cell suspension
6. Aliquot 100 µL of the cell suspension into each well of the 9 individual 24-well G-Rex plates per donor
7. Add 7 mL appropriate media from **Step 2** into the corresponding wells of each plate and incubate in standard tissue culture conditions

Harvest and analyze cells

8. Feed and harvest plates at appropriate time points
    a. Harvest the day 3 plates, count the cells and stain with the PBMC flow cytometry panel
    b. Feed day 7 and day 10 plates on day 5 by aspirating 5.25 mL of media from each well and replacing it with 5.25 mL of fresh, cytokine-supplemented media
    c. Harvest the day 7 plates, count the cells, and stain with the PBMC flow cytometry panel

9. On the last day of the experiment, harvest day 13 plates and split the cell population into 3 groups: cells for counting, cells for surface staining, and cells for function testing and intracellular staining
    a. Count cells and stain for surface markers as with previous time points

10. Prepare the function test
    a. Plate approximately $2x10^6$ cells/well into duplicate 96-well round-bottom lidded plates (one will be the unstimulated control)
    b. Stain both plates for CD107a
    c. Stimulate 1 plate with staphylococcal enterotoxin B (SEB)
    d. Incubate both plates for 2 hours in standard tissue culture conditions
    e. Add protein transport inhibitor to both plates and incubate for 4 hours

11. Initiate intracellular staining
    a. Fix and permeabilize the cells
    b. Stain cells for intracellular markers and cytokines
    c. Run flow cytometry

RESULTS

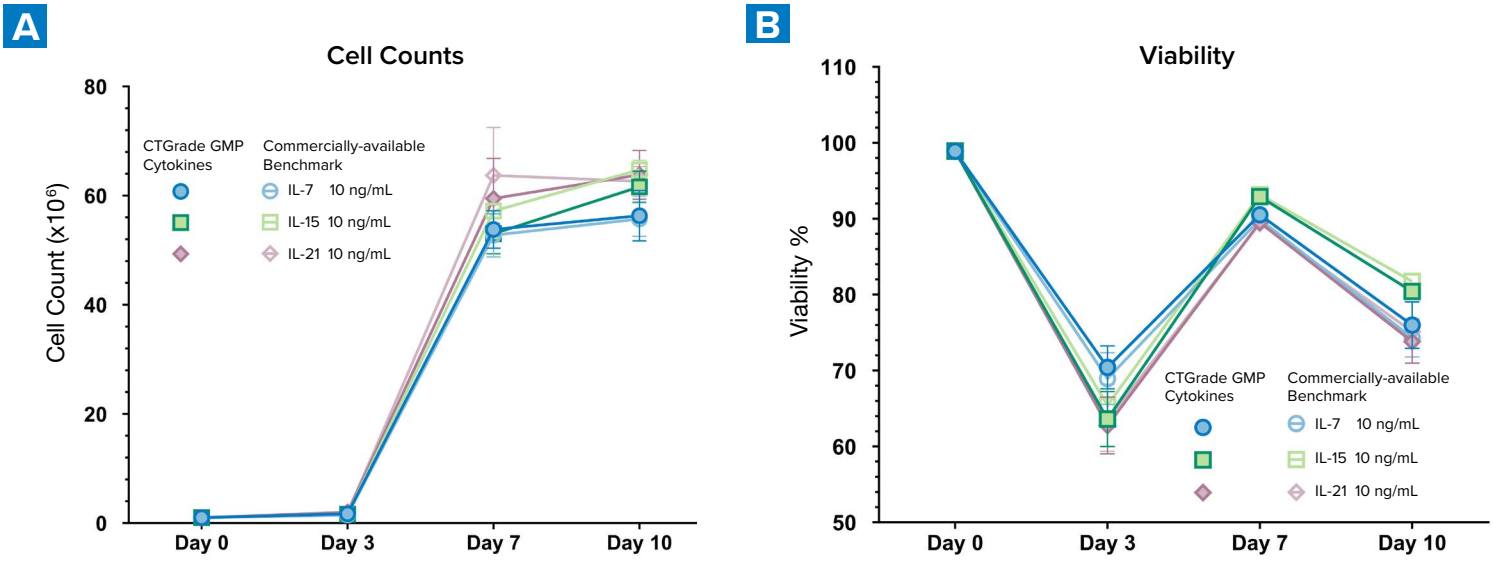


Figure 1. Fresh human PBMCs show robust expansion and high viability in PRIME-XV T Cell CDM supplemented with CTGrade GMP rh IL-7, IL-15, or IL-21 + IL-2_{C126S}. (A) By Day 10, all CTGrade GMP rh IL-containing conditions had comparable levels of expansion, with slightly less growth seen in lower concentrations of CTGrade GMP rh IL-7 and rh IL-15 (not shown). **(B)** The Day 3 drop in viability is commonly seen due to activation-induced apoptosis and is followed by complete recovery on Day 7. Cell viability remains above 75% over the course of the culture, and is equally supported by all concentrations and sources of interleukins. Results are representative of 3 donors, 3 lots, and 2 different concentrations of CTGrade GMP rh IL-7, IL-15, and IL-21 run in triplicates per condition.

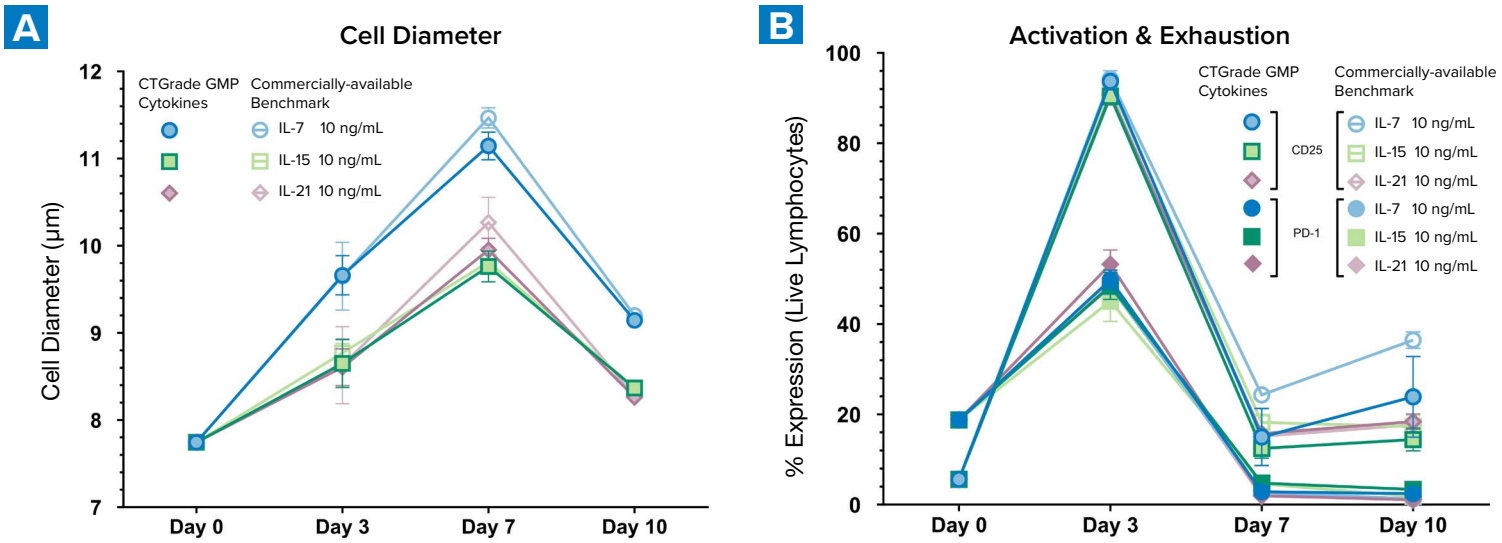
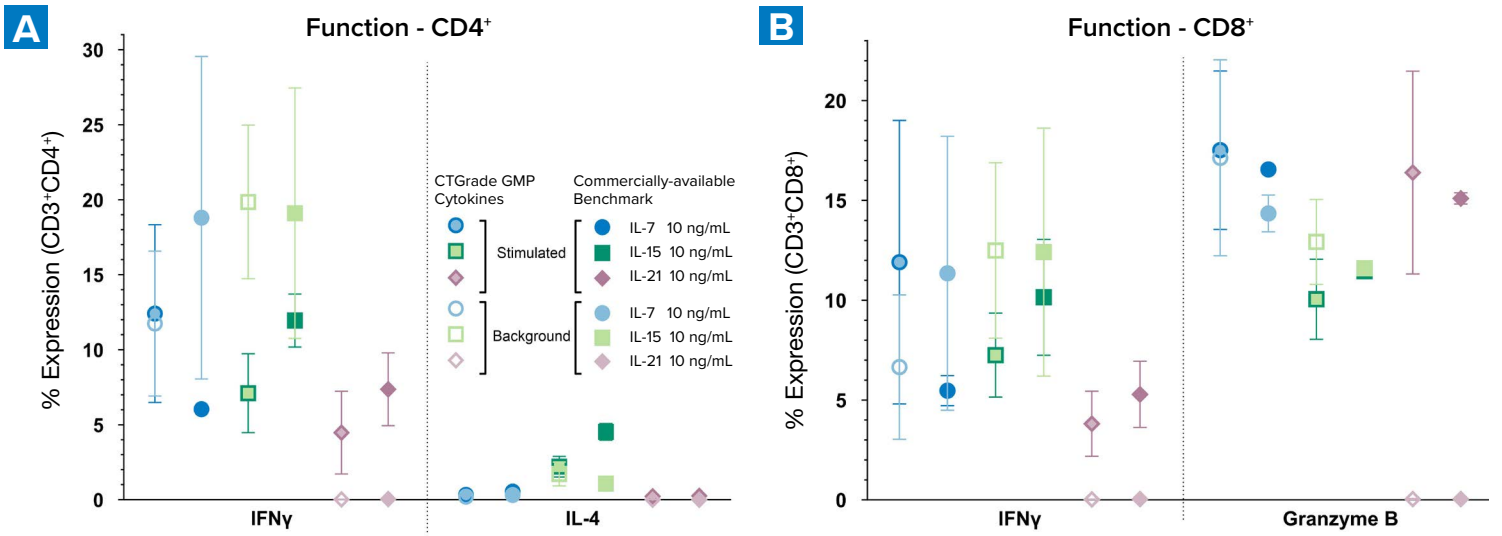


Figure 2. Fresh human PBMCs show healthy activation kinetics in PRIME-XV T Cell CDM supplemented with CTGrade GMP rh IL-7, IL-15, or IL-21 + IL-2$_{C126S}$. (A) The increase in cell diameter post-activation is more pronounced in conditions containing IL-7, but otherwise follows expected kinetics. **(B)** The Day 3 drop in viability is accompanied by an increase in PD-1, which decreases to negligible levels by Day 7 post-expansion. The activation marker CD25 drops off by the end of the first week of expansion and remains constant through Day 10. Results are representative of 3 donors, 3 lots, and 2 different concentrations of CTGrade GMP rh IL-7, IL-15, and IL-21 run in triplicates per condition.



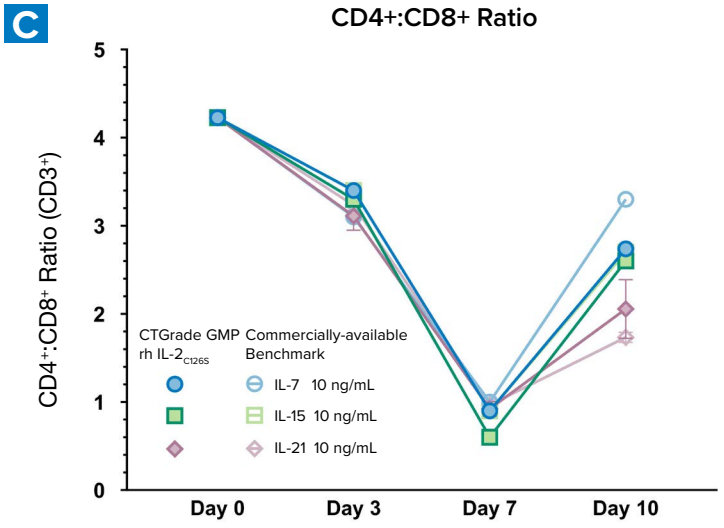


Figure 3. Cells retain a physiologically relevant ratio of helper to cytotoxic cells throughout expansion, and CTGrade GMP rh IL-2$_{C126S}$ is necessary to respond to supernatigen stimulation. The expression of **(A)** CD4+ and **(B)** CD8+ immunomodulatory cytokines is higher at baseline in conditions with exclusively CTGrade GMP rh IL-7 or IL-15. Only the CTGrade GMP rh IL-2$_{C126S}$-containing conditions showed an upregulation of functional markers following superantigen stimulation. **(C)** Though the CD4+:CD8+ ratio drops in the middle of the culture, it partially recovers by Day 10, indicating continued survival and proliferation of both T cell subsets. Results are representative of 3 donors, 3 lots, and 2 different concentrations of CTGrade GMP rh IL-7, IL-15, and IL-21 run in triplicates per condition.

DISCUSSION

Though exact results will vary depending on the donor-to-donor variation, CTGrade rh GMP IL-2_{C126S} at all tested concentrations supports activation and strong expansion of T cells in PRIME-XV T Cell CDM. Resultant cells show minimal exhaustion markers and robust activity when stimulated with a superantigen, while maintaining a physiologically relevant $CD4^+$:$CD8^+$ ratio. The combination of CTGrade GMP rh IL-2_{C126S} and PRIME-XV T Cell CDM provides a chemically defined, animal component-free environment for healthy T cells. This significantly reduces variables in the production of cell therapies, unknown contaminants, and risks to downstream applications in humans.

CTGrade GMP rh IL-7 and IL-15, when used exclusively at both tested concentrations, support robust expansion of T cells in PRIME-XV T Cell CDM with a variety of donors. Similar expansion is observed when combining CTGrade GMP rh IL-21 and IL-2_{C126S}. Resultant cells show minimal exhaustion markers and healthy activation kinetics, while maintaining a physiologically relevant $CD4^+$:$CD8^+$ ratio. Though cells expanded in CTGrade GMP rh IL-7 or IL15 alone did not show a significant response to superantigen stimulation, these cytokines are well-documented to complement each other in a variety of combinations, which may be investigated on an application-specific basis. Despite the variability of exact results due to donor-to-donor variation, CTGrade GMP rh IL-7, IL-15, and IL-21, along with PRIME-XV T Cell CDM, provide a recombinant human IL-2_{C126S} -independent, chemically defined, animal component-free environment for the activation and expansion of healthy T cells for cell therapy use. The use of these recombinant proteins significantly reduces variables in production, unknown contaminants, and risks to downstream applications in humans.

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