PRIME-XV T Cell CDM

PRIME-XV T Cell CDM is a ready-to-use chemically-defined, animal component-free medium. It is optimized and designed for the culture of T cells of human origin and recommended for use in the cultivation and expansion of human T lymphocytes. The performance of this medium was assessed on CD3+ T lymphocytes derived from human peripheral blood mononuclear cells (PBMCs) in plates, flasks, and G-Rex culture vessels. PRIME-XV T Cell CDM is intended to be used with cytokine supplements for the ex-vivo culture of T human lymphocytes. The cytokine cocktail used depends on the experimental requirements of each user.

Catalog #	Product	Size
91154	PRIME-XV T Cell CDM	1 L liquid Additional package sizes are available upon request

Intended Use

For research or further manufacturing purposes. Not for injection or diagnostic procedures.

Quality Assurance

All quality control test results are reported on a lot specific Certificate of Analysis which is available at www.irvinesci.com or upon request.

Storage Instructions and Stability

Handle using aseptic techniques to avoid contamination. Store at 2-8˚C and protect from light until ready to use. This product is stable at 2-8˚C, under original packaging, for 14 months from the date of manufacture for 1 L PET bottles. Once opened, the product should be stored at 2-8˚C in the dark and used within 4 weeks. Do not use after the assigned expiration date. Not validated for use beyond the unopened expiry shelf life. Please refer to the Safety Data Sheet for information regarding hazards and safe handling practices.

Precautions

This product is for research use or further manufacturing use only. Not for injection or diagnostic procedures. The safety and efficacy of this product in diagnostic or other clinical uses has not been established. This reagent should not be used beyond twelve months indicated in the storage instructions. Please refer to the Safety Data Sheet for information regarding hazards and safe handling practices.

Directions for Use

The following protocol is optimized for the expansion of activated $CD3^+$ T lymphocytes derived from peripheral blood mononuclear cells (PBMCs) using PRIME-XV T Cell CDM in tissue culture plates, flasks, and G-Rex.

PROTOCOL FOR T CELL EXPANSION IN TISSUE CULTURE PLATES AND FLASKS

Plate coating for T cell activation

1. Coat 12-well tissue culture plates with 1 µg/mL (143 ng/cm^2) each of anti-human CD3 (Biolegend® Clone OKT3, Cat# 317348) and anti-human CD28 (Biolegend® Clone CD28.2, Cat# 302944) antibodies for 2 hours at 37˚C or overnight at 2-8˚C with Parafilm® to prevent evaporation
2. Aspirate coating solution.
3. Wash twice with PBS before the addition of cells.

T cell preparation

4. Equilibrate sufficient volume of PRIME-XV T Cell CDM to 37˚C for at least 15 minutes before use.
   Note: To avoid temperature cycling, determine the total volume needed before equilibration.
5. Thaw a fresh vial of cryopreserved PBMCs by gently stirring the vial in a 37˚C water bath for 1 minute.
6. Carefully transfer entire content of the vial into a 15 mL conical tube containing pre-warmed 10 mL PRIME-XV T Cell CDM.
7. Spin cells down at 300 g for 5 minutes.
8. Carefully aspirate supernatant, leaving a minimum volume of media covering the cell pellet.
9. Supplement PRIME-XV T Cell CDM with the equivalent of 200 IU/mL IL-2.
10. Resuspend cell pellet with the supplemented PRIME-XV T Cell CDM and transfer cells onto the coated plate at a seeding density of 1x10^6 cells/mL.
11. Incubate the cells at 37˚C and 5% CO_2.
12. Count cells every two to three days and dilute to a cell density of 0.5x10^6 cells/mL with PRIME-XV T Cell CDM containing the equivalent of 200 IU/mL IL-2, transferring the culture to larger vessels as needed to accommodate for increased volume. Refer to the table below for the range of cell densities in cells/cm^2.

Vessels	Cells/mL (x10^6)	Cells (x10^6) /cm^2		Volume (mL)		Surface Area (cm^2)
		Min	Max	Min	Max	
12-well plate	0.5	0.143	0.286	1	2	3.5
6-well plate		0.052	0.156	1	3	9.6
T-25 flask		0.060	0.100	3	5	25
T-75 flask		0.053	0.100	8	15	75
T-175 flask		0.100	0.151	35	53	175
T-225 flask		0.100	0.151	45	68	225

T cell harvest

13. Gently pipette the cells to detach them from the vessel.
14. Wash with temperature equilibrated PRIME-XV T Cell CDM.
15. Cells are ready for analysis, banking, or re-plating.

PROTOCOL FOR T CELL EXPANSION IN 24-WELL G-REX VESSELS

Day 0: T cell plating and activation

1. Equilibrate sufficient volume of PRIME-XV T Cell CDM to 37˚C for at least 15 minutes before use
   Note: To avoid temperature cycling, determine the total volume needed before equilibration.
2. Thaw a frozen vial of cells by gently stirring the vial in a 37˚C water bath for 1 minute. Alternatively, use freshly isolated or harvested cells.
3. Carefully transfer entire contents of the vial into a 15 mL conical tube containing 10 mL PRIME-XV T Cell CDM.
4. Spin cells down at 300 g for 5 minutes.
5. Carefully aspirate supernatant, leaving a minimum volume of media covering the cell pellet.
6. Supplement appropriate volume of PRIME-XV T Cell CDM with the equivalent of 200 IU/mL IL-2.
7. Seed 1 x10^6 PBMCs/well in 7 mL of complete media.
8. Add 1 µg/mL each of anti-human CD3 (Biolegend® Clone OKT3, Cat# 317348) and anti-human CD28 (Biolegend® Clone CD28.2, Cat# 302944) antibodies.
9. Incubate cells at 37˚C and 5% CO_2.

Day 3: Continue stimulation

10. Supplement each well with an additional 200 IU/mL IL-2.

Day 5: Media exchange

11. Remove 5.25 mL of spent media by slowly pipetting from the top edge of the well down, carefully avoiding accidental aspiration of cells.
12. Gently swirl or resuspend the remaining liquid to evenly disperse cells for cell count.
13. Add 5.5 mL fresh media supplemented with 200 IU/mL IL-2.

Day 7: Continue stimulation

14. Supplement each well with an additional 200 IU/mL IL-2.

Day 10: Media exchange

15. Remove 5.25 mL of spent media by slowly pipetting from the top edge of the well down, carefully avoiding accidental aspiration of cells.
16. Gently swirl or resuspend the remaining liquid to evenly disperse cells for cell count.
17. Add 5.5 mL fresh media supplemented with 200 IU/mL IL-2.

Day 12: Continue stimulation

18. Supplement each well with an additional 200 IU/mL IL-2.

Day 14: Harvest Cells

19. Remove 5.25 mL of spent media by slowly pipetting from the top edge of the well down, carefully avoiding accidental aspiration of cells.
20. Gently swirl or resuspend the remaining liquid to evenly disperse cells for cell count.
21. Harvest cells in remaining volume.

Data

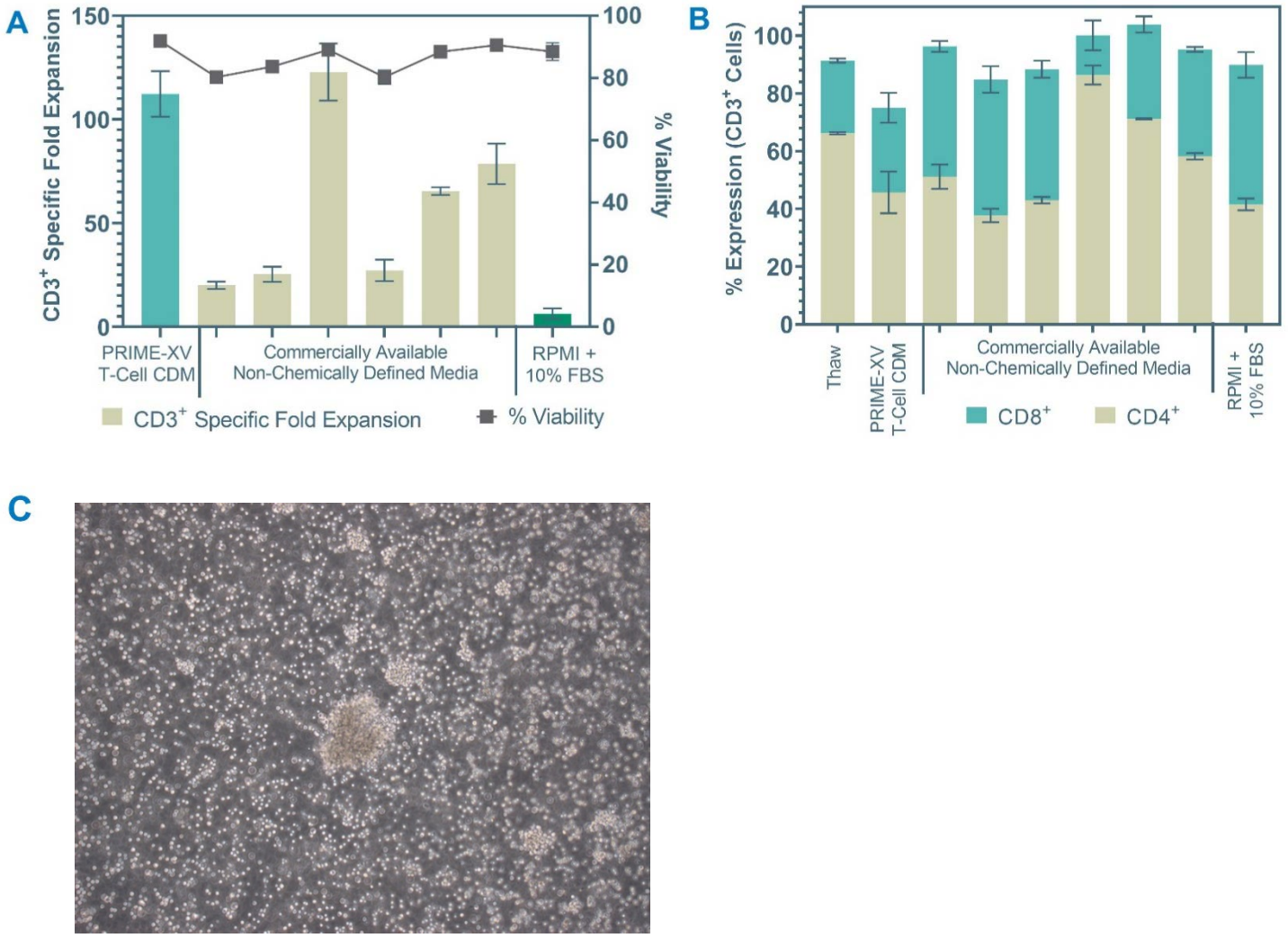

Figure 1. Expansion of T Cells in PRIME-XV T Cell CDM Compared to Commercially Available Non-Chemically Defined Media. Human PBMCs were activated and cultured in PRIME-XV T Cell CDM or commercially available non chemically-defined expansion media supplemented with 200 IU/mL IL-2. These results are representative of three healthy donors, run in triplicate. Day 10 data is featured in this figure because it represents the peak of exponential expansion (A) After 10 days, viability and fold expansion of $CD3^+$ T cells were quantified. (B) Flow cytometry analysis demonstrated the ratios of $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells on day 10 to the initial PBMC ratios at thaw. (C) Image of activated cells cultured in PRIME-XV T Cell CDM on day 10 (100X magnification).

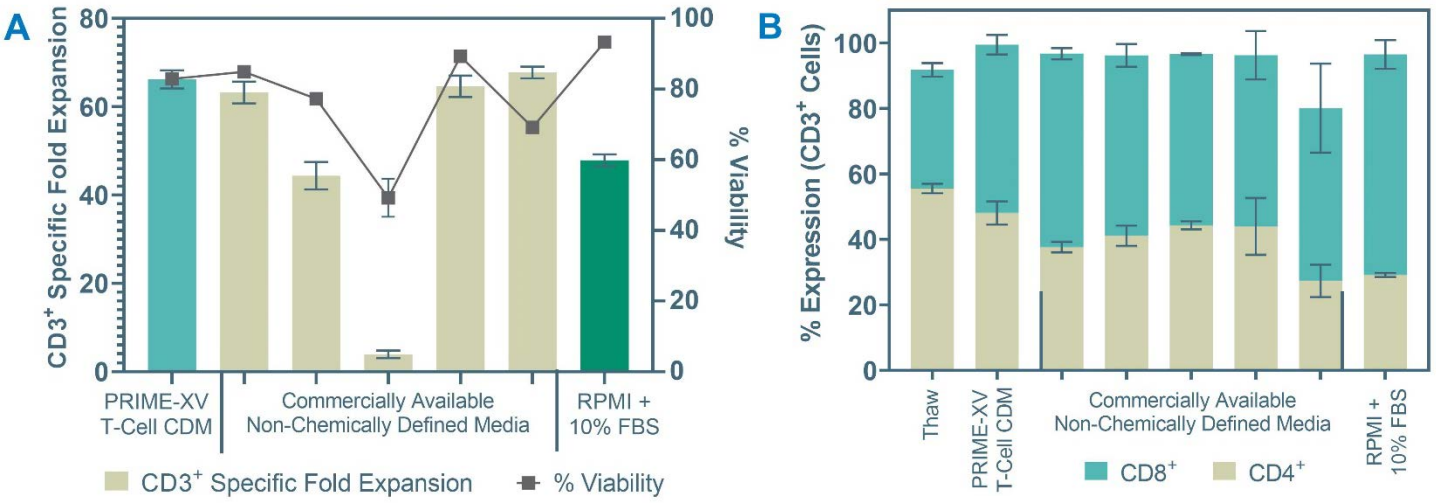

Fig 2. Expansion of T Cells in PRIME-XV T Cell CDM Compared to Non-Chemically Defined Media in the G-Rex Cell Culture Device. $CD3^+$ T cells derived from human peripheral blood mononuclear cells (PBMC), were activated with soluble anti-human CD3 and anti-human CD28 antibodies. These results are representative of three healthy donors, run in triplicate. Day 10 data is featured in this figure because it represents the peak of exponential expansion. (A) After 10 days of culture in various media supplemented with 200 IU/mL IL-2, cells were harvested and analyzed for viability and fold expansion. (B) Flow cytometry analysis demonstrated the ratios of $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells on day 10 to the initial PBMC ratios at thaw.

Related Products

Catalog #	Product	Size
9240	1X PBS, Dulbecco's Phosphate Buffered Saline	100 mL, 500 mL, 1L
95118	Recombinant Human IL-2 ACF	10 µg
95113	Recombinant Human IL-3 ACF	10 µg
95114	Recombinant Human IL-4 ACF	20 µg
91139	PRIME-XV FreezIS	10 mL, 100 mL

Technical support

CONTACT US

For more information or assistance contact Customer Service at:

- Email: fisitmrequest@fujifilm.com
- Direct line: +1 800 577 6097

WEBSITE RESOURCES

Visit the website at www.irvinesci.com for technical resources and information including:

- Safety Data Sheets (SDS)
- COAs (when available)
- FAQs
- Product literature
- Complete list of offices and contact information by country

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