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 lentiviral transduction, 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 G-Rex culture vessels and Quantum bioreactors. 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, bottle 1 L liquid, bag 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 https://fujifilmbiosciences.fujifilm.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. Unopened liquid medium is stable for 18 months from date of manufacture. 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 the date of expiry. The product may develop some protein precipitate during storage or in culture over time. This protein precipitate is not known to have an effect on product performance. 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 G-Rex.

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 ($0.5x10^6$ cells/cm^2) in 7 mL of complete media.
8. Add 1 µg/mL each of anti-human CD3 and anti-human CD28 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.

PROTOCOL FOR LOW-SEED T CELL EXPANSION IN QUANTUM BIOREACTOR

Materials (Per machine)

- 1x Quantum hollow fiber bioreactor cell expansion set (Terumo BCT Cat# 21000)
- Cell expansion set priming: Minimum 2 L PBS (FLBI Cat# 9240) in Quantum-compatible bags
- Intracapillary media (IC): Minimum 3.5 L complete T-cell CDM (FLBI Cat# 91154) + 200IU/mL IL-2 (FLBI Cat# 95118) in Quantum-compatible bags
- Extracapillary media (EC): Minimum 11 L T-cell CDM without IL-2 in Quantum-compatible bags
- Aluminum foil or other cover to protect bags from light
- $30x10^6$ PBMCs
- Activation: Commercially available T-cell activation beads
- Thaw cells: T-175 flask (Corning Cat# 430641U)
- Minimum 1 cell inlet bag (Terumo BCT Cat# 21028)
- Minimum 4 waste bags (Terumo BCT Cat# 21030)

Additional Materials

- T-Seal Mobile or other compatible sealer (Terumo BCT Cat# T5460)
- TSCD-Q Sterile Tubing Welder (Terumo BCT Cat# 92003)
- 5-mL luer-lock syringes
- 50 mL conical tubes
- Alcohol swabs
- Scissors

Day -1: Rest thawed cells

1. If thawing cells, let rest overnight in culture flask and complete culture media at 37˚C 5% CO_2.

Day 0: Prepare cell expansion set and load cells

2. Load cell expansion set as specified in the Quantum Cell Expansion System Operator's Manual (QCESOM) Section 7-2.
3. Prime cell expansion set (QCESOM 7-10)
4. Separate the tube lines (QCESOM 7-11)
5. Attach EC media bag to EC line and run IC/EC Washout (QCESOM Section 9-5)
   Use the following settings for the washout:

IC inlet	EC media
IC inlet rate (mL/min)	100
IC circ rate (mL/min)	-17
EC inlet	EC media
EC inlet rate (mL/min)	148
EC circ rate (mL/min)	-1.7
Outlet	IC and EC outlet
Rocker	In motion (-90˚, 180˚, 1 sec)
Stop condition	Exchange (2.5 IC volume, 2.5 EC volume)

6. Condition media (QCESOM Section 8-6)
   Use the following settings for the conditioning:

IC inlet	None
IC inlet rate (mL/min)	0
IC circ rate (mL/min)	20
EC inlet	None
EC inlet rate (mL/min)	0
EC circ rate (mL/min)	30
Outlet	EC outlet

Rocker	Stationary (0˚)
Stop condition	Manual (≥ 10 min)

7. As the conditioning program is running, activate $30x10^6$ PBMCs with a 1:1 ratio of commercially available T-cell activation beads for approximately ten minutes, gently agitating the container at room temperature for the duration. Perform cell counts and save a small untreated sample for flow cytometry analysis.
8. Load $30x10^6$ activated cells, 50 mL IC media, and at least 10 mL air into a cell inlet bag.
9. Attach appropriate bags to their corresponding lines (IC media, EC media, Cell) and replace the waste bag with a fresh one. Replace waste bags and EC/IC media bags as needed throughout culture by pausing the program for the duration of the replacement process.
10. Take a sample of media from the EC sample port for baseline metabolite readings (QCESOM Section 5-7).
11. Set up and run Custom Program 1 for T-cell loading and feeding (QCESOM Section 13-2)
    Use the following settings for Custom 1:

	Step 1	Step 2	Step 3	Step 4
IC inlet	Cell	IC media	IC media	IC media
IC inlet rate (mL/min)	50	50	80	0.1
IC circ rate (mL/min)	0	0	-40	-0.1
EC inlet	None	None	None	None
EC inlet rate (mL/min)	0	0	0	0
EC circ rate (mL/min)	30	30	30	30
Outlet	IC outlet	IC outlet	EC outlet	EC outlet
Rocker	In motion (-90˚, 180˚, 1 sec)	In motion (-90˚, 180˚, 1 sec)	In motion (-90˚, 180˚, 1 sec)	Stationary (0˚)
Stop condition	Empty bag	IC volume (50 mL)	IC volume (310 mL)	Manual (2880 min)

12. Collect daily samples of EC media from the EC sampling port for metabolite analysis, taking note of the time these samples were taken (QCESOM Section 5-7). This continues for the duration of the culture.

Day 2: Begin feeding culture

13. Set up Custom Program 2 for one daily cell redistribution and feed (QCESOM Section 13-2).
    Use the following settings for Custom 2:

	Step 1	Step 2	Step 3
IC inlet	None	EC media	IC media
IC inlet rate (mL/min)	0	50	0.1
IC circ rate (mL/min)	300	-30	-0.1
EC inlet	None	None	None

EC inlet rate (mL/min)	0	0	0
EC circ rate (mL/min)	100	30	100
Outlet	EC outlet	EC outlet	EC outlet
Rocker	In motion (-90˚, 180˚, 1 sec)	In motion (-90˚, 180˚, 1 sec)	Stationary (0˚)
Stop condition	Time (4 min)	IC volume (150 mL)	Manual (1440 min)

NOTE: Pause the program 3.5 minutes into Step 1 of Program 2 to take EC samples. Once samples are taken, program may be resumed.

14. Restart Custom Program 2 on days 3, 4, and 5, taking EC samples as described in Step 13.

Day 5

15. Collect IC sample (QCESOM Section 5-5) alongside EC sample (QCESOM Section 5-7) and run flow cytometry and counts on the cells.
16. Adjust IC inlet and circulation rates as necessary to maintain appropriate glucose and lactate levels in culture.

Day 6

17. Set up Custom Program 3 for two daily cell redistributions and feeds (QCESOM Section 13-2). Use the following settings for Custom 3:

	Step 1	**Step 2**	**Step 3**
IC inlet	None	EC media	IC media
IC inlet rate (mL/min)	0	50	0.4
IC circ rate (mL/min)	300	-30	-0.2
EC inlet	None	None	EC media
EC inlet rate (mL/min)	0	0	0.8
EC circ rate (mL/min)	300	30	300
Outlet	EC outlet	EC outlet	EC outlet
Rocker	In motion (-90˚, 180˚, 1 sec)	In motion (-90˚, 180˚, 1 sec)	Stationary (0˚)
Stop condition	Time (4 min)	IC volume (150 mL)	Time (720 min)

	Step 4	**Step 5**	**Step 6**
IC inlet	None	EC media	IC media
IC inlet rate (mL/min)	0	50	0.4
IC circ rate (mL/min)	-300	-30	-0.2
EC inlet	None	None	EC media

EC inlet rate (mL/min)	0	0	0.8
EC circ rate (mL/min)	100	30	300
Outlet	EC outlet	EC outlet	EC outlet
Rocker	In motion (-90˚, 180˚, 1 sec)	In motion (-90˚, 180˚, 1 sec)	Stationary (0˚)
Stop condition	Time (4 min)	IC volume (150 mL)	Manual (720 min)

NOTE: Pause the program 3.5 minutes into Step 1 of redistribution to take EC (QCESOM Section 5-7) and IC (QCESOM Section 5-5) samples. Once samples are taken, program may be resumed.

18. Repeat Custom 3 on days 7 and 8, taking EC and IC samples as described in Step 17. Perform spent media analysis on the EC samples, and run cell counts and flow cytometry on the IC samples.
19. Adjust EC inlet rate as necessary to maintain appropriate glucose and lactate levels in culture, keeping in mind media consumption rates and waste bag fill rates.

Day 9

20. Set up Custom Program 4 for cell harvest (QCESOM Section 13-2).
    Use the following settings for Custom 4:

	Step 1	**Step 2**
IC inlet	None	EC media
IC inlet rate (mL/min)	0	100
IC circ rate (mL/min)	300	-20
EC inlet	None	EC media
EC inlet rate (mL/min)	0	60
EC circ rate (mL/min)	300	30
Outlet	EC outlet	Harvest
Rocker	In motion (-90˚, 180˚, 1 sec)	In motion (-90˚, 180˚, 1 sec)
Stop condition	Time (4 min)	IC Volume (400 mL)

21. Run cell counts, spent media analysis, and flow cytometry on the final cell harvest.

Directions for Use

The following protocol details lentiviral transduction and expansion of peripheral blood mononuclear cells (PBMCs) in PRIME-XV T Cell CDM using the G-Rex culture system.

INTRODUCTION

Specialized viral vectors are commonly used to genetically modify immune cells for cell therapy applications. The vast variability between primary human immune cells from different donors presents a roadblock for universally effective transduction. Transduction efficiency is likewise negatively impacted by the relatively large size of gene inserts and the necessity for expedient processing. PRIME-XV T Cell CDM is a chemically-defined, animal component-free medium that supports transduction and expansion of human PBMCs without requiring spinoculation or transduction enhancers. Manufactured under cGMP conditions with lot-to-lot consistency, PRIME-XV T Cell CDM provides a favorable environment for lentiviral transduction and subsequent robust expansion of T cells.

BULK ACTIVATION

1. If thawing rather than using freshly harvested cells, rest cells overnight in PRIME-XV T Cell CDM + 200 IU/mL IL-2 to allow them to recover from cryopreservation
    a. 37˚C, 5% CO_2, humidified incubator
2. Count cells prior to activation
    a. A minimum of 0.125 x 10^6 cells per cm^2 of G-Rex culture surface area is needed for the experiment (See Table 1 for cell count guide)
    b. Allow for cell loss during activation by activating more cells than necessary for experiment setup

    NOTE: To generate data below, FLBI used fresh PBMCs (StemExpress #PBMNC300F)
3. Activate the cells with αCD3 and αCD28 antibodies in tissue culture flask
    a. Bound antibodies
        a. Prepare a solution of 1 µg/mL αCD3 and 1 µg/mL αCD28 antibodies in PBS
        b. Coat culture surface of flask for 2 hours at 37˚C or overnight at 2-8˚C
        c. If activating overnight, wrap the flask lid in Parafilm to prevent evaporation
        d. Aspirate coating solution prior to plating the cells
        e. Plate cells at a density between 1 x 10^6 cells/mL and 5 x 10^6 cells/mL for activation
        f. Activation may be done for a minimum of 6 hours, up to 24 hours
    b. Soluble antibodies
        a. Plate cells at a density between 1 x 10^6 cells/mL and 5 x 10^6 cells/mL for activation in cell culture flask
        b. Add 1 µg/mL αCD3 and 1 µg/mL αCD28 antibodies to cell suspension
        c. Activation may be done for a minimum of 6 hours, up to 24 hours
4. After cell activation, harvest the cells in to a sterile conical tube and spin the cells down at 300g for 5 minutes
5. Resuspend desired amount of cells in a conical tube at 0.5 x 10^6 cells/mL in PRIME-XV T Cell CDM + 200 IU/mL IL-2
    a. Set aside an aliquot for the negative (no virus) control

LENTIVIRAL TRANSDUCTION

NOTE: Titration of lentivirus may be necessary for individual applications. Transduction efficiency varies greatly between PBMC donors and specific lentiviral constructs. Provided concentrations have been optimized for PBMC transduction with a GFP reporter lentivirus using the PGK promoter (Cellomics Technology #PLV-10077).

6. Add lentivirus to cell suspension prepared in Step 5 at optimal multiplicity of infection (MOI) for your application
    a. FLBI uses MOI = 5
7. Aliquot 0.25 mL/cm^2 lentivirus & cell suspension mixture prepared in Step 6 to each vessel (See Table 1 for transduction volume)
    a. The negative control set aside in Step 3 is a recommended condition
    b. Prepare duplicate or triplicate wells/vessels per condition
8. Incubate overnight in normal tissue culture conditions
    a. 37˚C, 5% CO_2, humidified incubator

9. The following day, fill the G-Rex wells or vessels to maximum capacity with PRIME-XV T Cell CDM + 200 IU/mL IL-2 (See Table 1 for maximum fill volume)
10. Incubate cell for an additional two days undisturbed in normal tissue culture conditions
    a. 37˚C, 5% CO_2, humidified incubator
11. Culture and expand cells as needed for up to 14 days. Recommended time points for spent media analysis (SMA), cell counts & viability tracking, and flow cytometry analysis are days 0, 3, 7, 10, and 14.

G-Rex Vessel	Min. cells per well or vessel (Steps 2 and 7)	Transduction volume (Step 7)	Maximum fill volume (Step 9)
24 Well Plate	0.25 x 10^6 cells/well	0.5 mL	8 mL
6 Well Plate	1.25 x 10^6 cells/well	2.5 mL	40 mL
6M Well Plate	1.25 x 10^6 cells/well	2.5 mL	100 mL
5M Open System & Closed System	0.625 x 10^6 cells/unit	1.25 mL	50 mL
10M Open System & Closed System	1.25 x 10^6 cells/unit	2.5 mL	100 mL
100M Open System & Closed System	12.5 x 10^6 cells/unit	25 mL	1000 mL
500M Open System & Closed System	62.5 x 10^6 cells/unit	125 mL	5000 mL

Table 1. Minimum cell counts, transduction volume, and culture volumes for transducing cells in G-Rex cell culture vessels.

Data

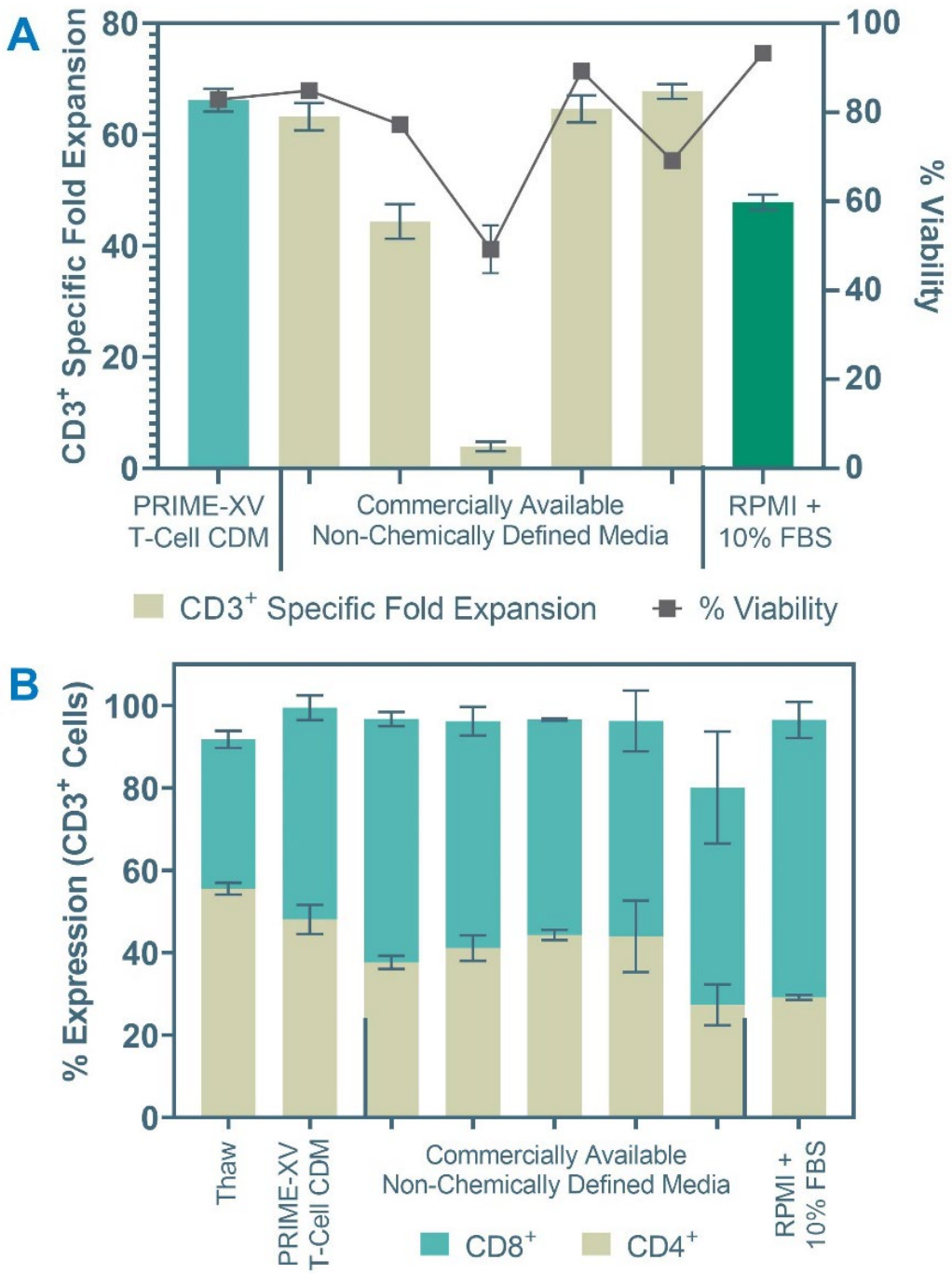


Fig 1. Expansion of T Cells in PRIME-XV T Cell CDM Compared to Non-Chemically Defined Media in the G-Rex Cell Culture Device. $CD3^+$ T cells derived from human peripheral blood mononuclear cells (PBMC), were activated with soluble anti-human CD3 and anti-human CD28 antibodies. These results are representative 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.

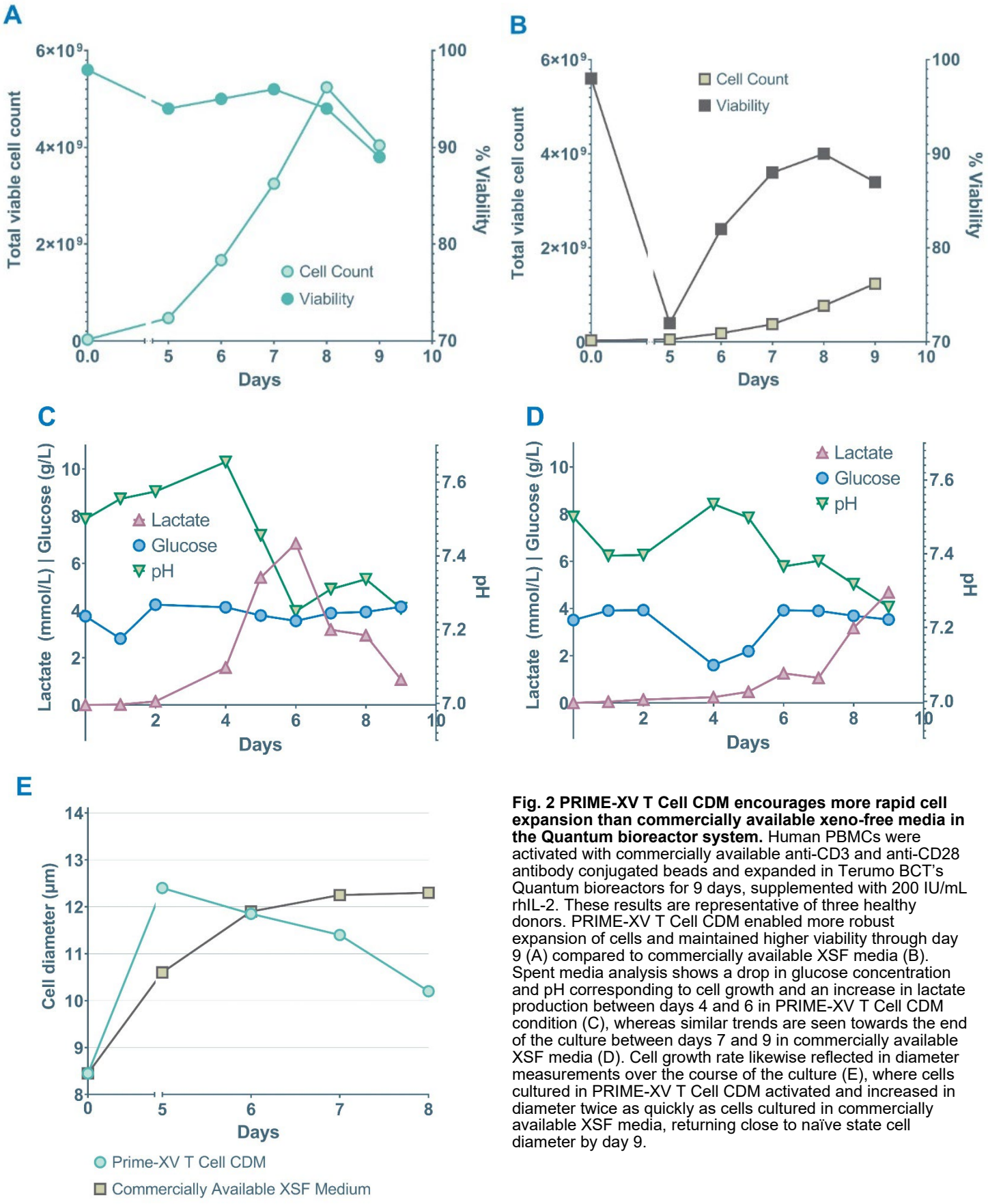


Fig. 2 PRIME-XV T Cell CDM encourages more rapid cell expansion than commercially available xeno-free media in the Quantum bioreactor system. Human PBMCs were activated with commercially available anti-CD3 and anti-CD28 antibody conjugated beads and expanded in Terumo BCT's Quantum bioreactors for 9 days, supplemented with 200 IU/mL rhIL-2. These results are representative of three healthy donors. PRIME-XV T Cell CDM enabled more robust expansion of cells and maintained higher viability through day 9 (A) compared to commercially available XSF media (B). Spent media analysis shows a drop in glucose concentration and pH corresponding to cell growth and an increase in lactate production between days 4 and 6 in PRIME-XV T Cell CDM condition (C), whereas similar trends are seen towards the end of the culture between days 7 and 9 in commercially available XSF media (D). Cell growth rate likewise reflected in diameter measurements over the course of the culture (E), where cells cultured in PRIME-XV T Cell CDM activated and increased in diameter twice as quickly as cells cultured in commercially available XSF media, returning close to naïve state cell diameter by day 9.

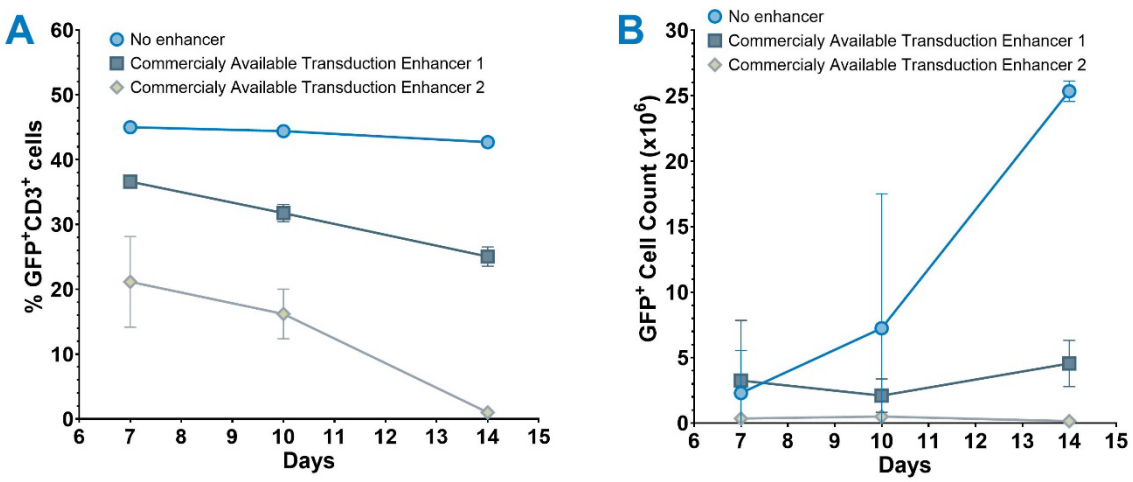


Figure 3. PRIME-XV T Cell CDM supports lentiviral transduction in the absence of commercially available transduction enhancers. (A) Human PBMCs transduced and expanded in PRIME-XV T Cell CDM + 200 IU/mL rhIL-2 without transduction enhancers maintain high level of GFP reporter expression at day 14 of culture. (B) The addition of commercially available transduction enhancers inhibited cell expansion in the second week post-transduction, yielding a lower total count of transduced GFP+ lymphocytes.

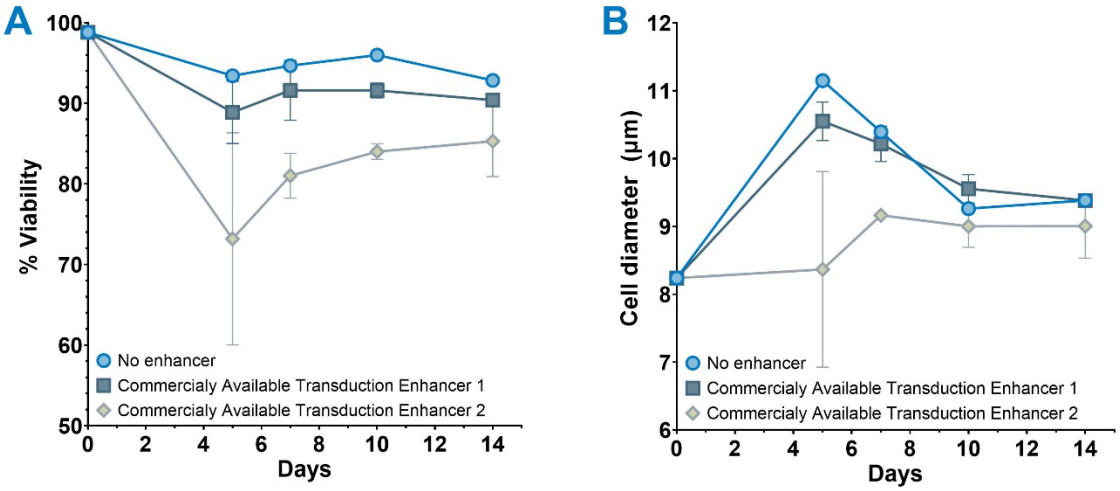


Figure 4. PRIME-XV T Cell CDM supports health of transduced cells for the culture duration. (A) Addition of transduction enhancers reduces viability of transduced human PBMCs. (B) Average cell diameter increases with T cell activation and returns to naïve state by day 14 of expansion.

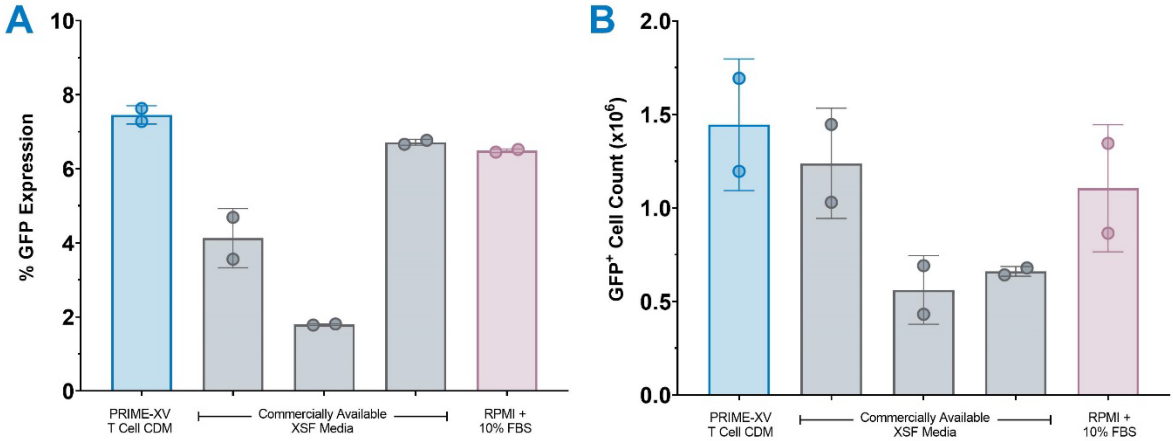


Figure 5. Enhancer-free transduction in PRIME-XV T Cell CDM is equal to or better than commercially available xeno-free media and FBS-supplemented RPMI. (A) Transduction efficiency and (B) total cell count of GFP reporter-positive human CD3+ T cells at day 14 of expansion are robust in the chemically defined medium, even in the absence of transduction enhancers.

Related Products

Catalog #	Product	Size
91140	PRIME-XV FreezIS DMSO-Free	10 mL, 100 mL
91139	PRIME-XV FreezIS	10 mL, 100 mL
9240	PBS 1X-Dulbecco's Phosphate Buffered Saline Solution-Liquid	100 mL, 500 mL, 1L
500-01	CTGrade rh IL-2 $_{C126S}$	50 µg, 100 µg, 1 mg
500-07	CTGrade rh IL-7	50 µg, 100 µg, 1 mg
500-16	CTGrade rh IL-10	50 µg, 100 µg, 1 mg
500-08	CTGrade rh IL-15	50 µg, 100 µg, 1 mg
500-09	CTGrade rh IL-21	50 µg, 100 µg, 1 mg
91165	BalanCD HEK293 media	1 L
91166	BalanCD HEK293 Feed	500 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 https://fujifilmbiosciences.fujifilm.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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