PRIME-XV T Cell Expansion XSFM

Catalog #	Product	Size
91141	PRIME-XV T Cell Expansion XSFM	1 L liquid

Intended Use

For research or further manufacturing use only.

Product Description

PRIME-XV T Cell Expansion XSFM is a xeno-free, serum-free medium optimized for the activation and expansion of human T lymphocytes. This medium contains gentamicin and requires additional cytokines according to desired applications.

Quality Assurance

All quality control test results are reported on a lot specific Certificate of Analysis (COA) which is available at https://fujifilmbiosciences.fujifilm.com/ or upon request.

Shipping

This product is shipped at 2-8°C. Upon receipt, store immediately at 2-8°C and protect from light.

Storage Instructions and Stability

Handle using aseptic techniques to avoid contamination. Unopened liquid medium is stable for 18 months from date of manufacture, as indicated on the label, when stored at 2-8°C and protected from light. PRIME-XV T Cell Expansion XSFM should be used within one month after opening when stored at 2-8°C and protected by light. PRIME-XV T Cell Expansion XSFM can be aliquoted and stored at or below -10°C in a manual defrost freezer for up to 3 months. Repeated freeze-thaw cycles should be avoided. When ready to use, thaw this medium overnight at 2-8°C in the dark. This product should be used within one month after thaw when stored at 2-8°C and protected by light. This product has not been validated for use beyond the unopened expiry shelf life.

Precautions

The safety and efficacy of this product in diagnostic or other clinical uses has not been established. Please refer to the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices. PRIME-XV T Cell Expansion XSFM should be handled as biohazardous and potentially infectious. Safe laboratory procedures should be observed and appropriate personal protective equipment should be worn when handling this medium. The acute and chronic effects of over-exposure to this product are unknown.

Directions for Use

The following protocol has been utilized for the activation and expansion of negatively selected $CD3^+$ T cells obtained from human peripheral blood and for human PBMCs with PRIME-XV T Cell Expansion XSFM. Further optimization may be required depending on the desired application.

Coating Procedure for T Cell Activation

This following is a guideline for T cell activation using anti-CD3 and anti-CD28 antibodies. Alternative approaches to T cell activation (e.g. beads, mitogens, activator antigens, or other T cell receptor antibodies) may be used. Types and amounts are dependent on the experimental design of each individual investigator.

1. Coat culture vessel with anti-human CD3 (clone UCHT1) and anti-human CD28 (clone CD28.2) antibodies at 1 µg/mL in PBS (PN# 9236) for 2 hours at 37°C or overnight at 2-8°C with Parafilm® to prevent evaporation.

2. Aspirate coating solution and discard.

3. Wash culture vessel twice with PBS (PN# 9236) before the addition of cells.

Recovery of Cryopreserved Cells

The following is a guideline based on the cryopreservation of negatively selected $CD3^+$ T cells obtained from human peripheral blood and for human PBMCs with PRIME-XV T Cell Expansion XSFM. Specific procedures provided by the respective cell supplier may also be utilized according to the tissue source and user preference and need.

1. Pre-warm sufficient amount of PRIME-XV T Cell Expansion XSFM in a 37°C water bath.

   NOTE: To avoid temperature cycling, determine the total volume needed before warming the media.

2. Carefully remove the frozen vial of T cells from liquid nitrogen and thaw by gently stirring the vial in a 37°C water bath for 1 minute.

3. Carefully transfer the entire content of the vial into a 15 mL conical tube containing 10 mL of pre-warmed PRIME-XV T Cell Expansion XSFM.

4. Spin cells down at 300 x g for 5 minutes.

5. Carefully aspirate supernatant leaving a minimum volume of media covering the cell pellet.

6. Supplement PRIME-XV T Cell Expansion XSFM with the equivalent of 200 IU/mL IL-2.

Expansion in tissue culture plates & flasks

1. Resuspend the cell pellet with the supplemented PRIME-XV T Cell Expansion XSFM and transfer cells onto the pre-coated culture vessel (see Coating Procedure) at a seeding density of 1 x 10^6 cells/mL (or according to suggested density recommendations from supplier).

2. Incubate the cells in a 37°C, 5% CO_2 humidified incubator.

3. Count cells every two to three days and dilute to a cell density of $0.5x10^6$ cells/mL with PRIME-XV T Cell Expansion XSFM 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

Expansion in 24-well G-Rex plates

1. Seed 1 $x10^6$ PBMCs/well ($0.5x10^6$ cells/cm^2) in 8 mL of complete media.

2. 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 or alternative T Cell activating reagents.

3. Incubate cells at 37˚C and 5% CO_2.

4. Supplement wells with 200IU/mL IL-2 on day 3 and every 4-5 days thereafter.

5. Perform a media exchange every 5 days of expansion.
    a. Remove 75% of spent media (~6mL) by slowly pipetting from the top edge of the well down, carefully avoiding touching the pipette tip to the gas permeable membrane and the accidental aspiration of cells.
    b. Gently swirl or resuspend the remaining liquid to evenly disperse cells for cell count.
    c. Add ~6 mL fresh media supplemented with 200 IU/mL IL-2.

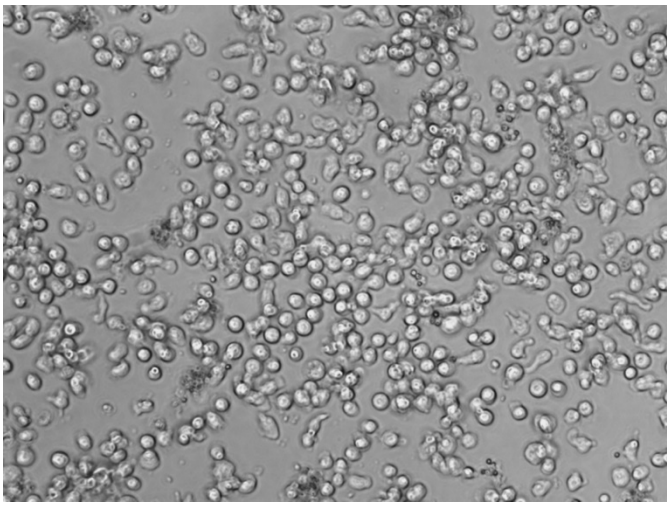
Harvesting of T cells

1. Gently pipette the cells to detach them from the vessel. A cell scraper may be used to gently dislodge adherent T cells.

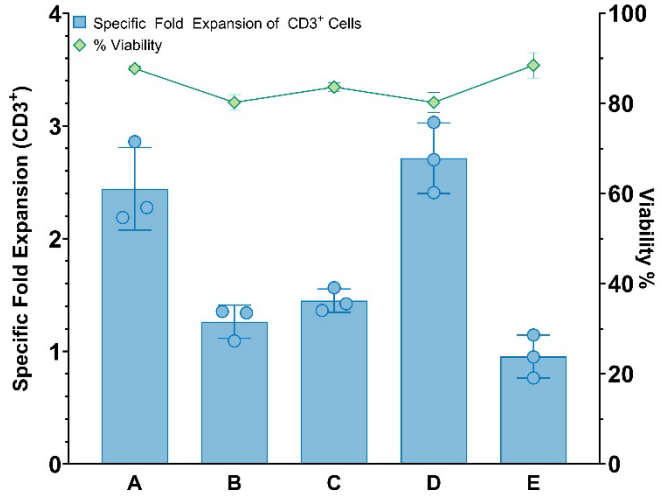
2. Wash with pre-warmed PRIME-XV T Cell Expansion XSFM.

3. Cells are ready for next desired application or analysis.

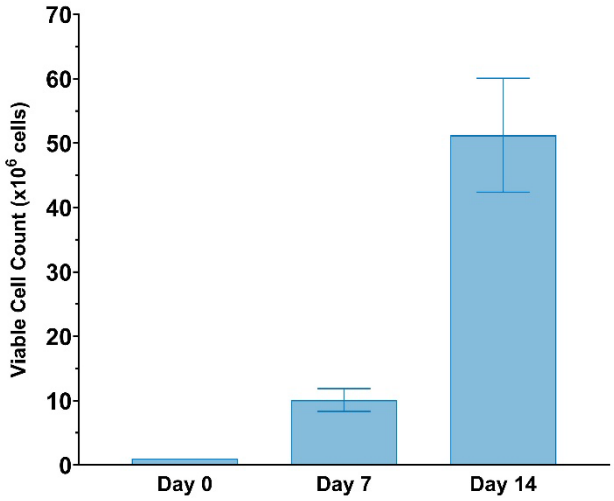
Sample Data



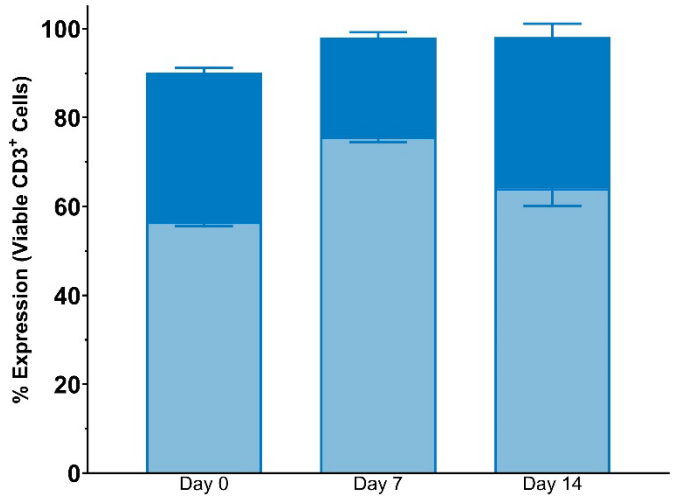
PRIME-XV T Cell Expansion XSFM maintains characteristic morphology of activated T cell blasts.
Naive $CD3^+$ T cells were cultured in IL-2 supplemented PRIME-XV T cell Expansion XSFM on anti-human CD3 (clone UCHT1) and anti-human CD28 (clone CD28.2) antibody coated plates at a seeding density of 0.5 x 10^6 cells/mL. Images were taken on day 2 of culture at 40X magnification.



PRIME-XV T Cell Expansion XSFM supports selective expansion of T Cells from PBMCs in static culture systems.
Human PBMCs were cultured in IL-2 supplemented PRIME-XV T Cell Expansion XSFM **(A)**, serum-containing medium **(E)**, and in four leading supplier's serum-free medium **(B through D)** on anti-human CD3 (clone UCHT1) and anti-human CD28 (clone CD28.2) antibody coated 12-well plates at a seeding density of 1 x 10^6 cells/mL. Cells were harvested, counted and stained with trypan blue for viability on the fifth day.

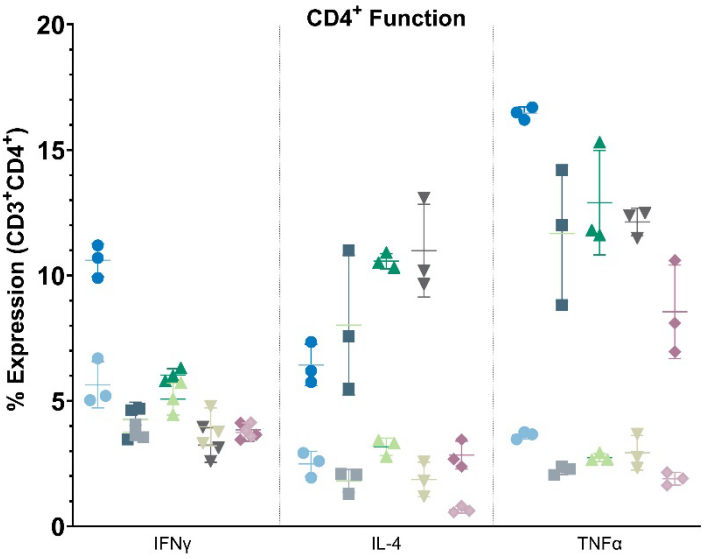


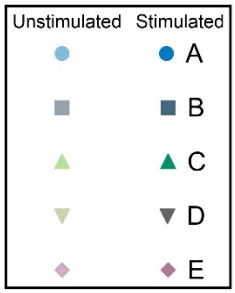
PRIME-XV T Cell Expansion XSFM supports selective expansion of T Cells from PBMCs in G-Rex culture systems.
PBMCs were seeded at 1x10^6 cells/well in IL-2 supplemented PRIME-XV T Cell Expansion XSFM with anti-human CD3 and anti-human CD28 T cell activation beads in 24-well G-Rex culture vessels. Cells were sampled, counted and stained with trypan blue for viability on the seventh and fourteenth days of culture. Results are representative of three different PBMC donors.

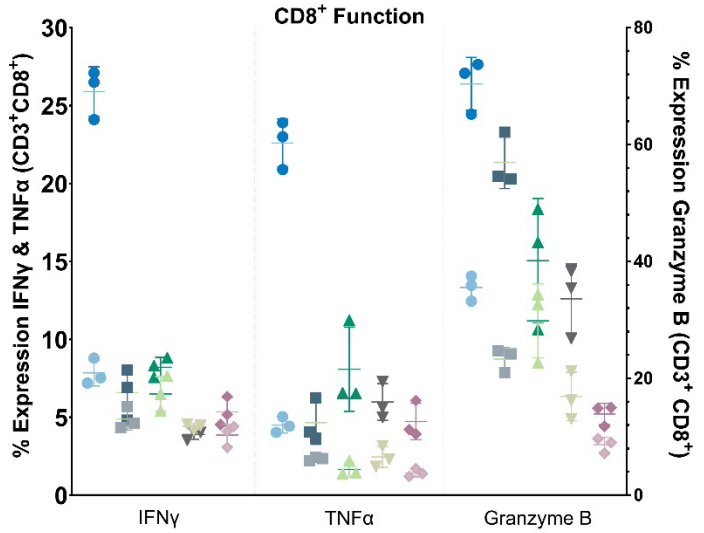


PRIME-XV T Cell Expansion XSFM maintains $CD4^+/CD8^+$ ratio in PBMCs.

PBMCs were cultured in IL-2 supplemented PRIME-XV T Cell Expansion XSFM with anti-human CD3 and anti-human CD28 T cell activation beads in 24-well G-Rex culture vessels. Phenotypic analyses were performed for the PBMCs after the initial thaw of the cells (Day 0) and after 7 and 14 days of culture in PRIME-XV T Cell Expansion XSFM. The cells were stained with APC-conjugated mouse anti-human CD3 (Clone UTHC1), PE-conjugated mouse anti-human CD8 (Clone SK1), and PerCP-Cy5.5 conjugated mouse anti-human CD4 (Clone OKT4). $CD4^+CD3^+$ cells are light blue (bottom stacked bar) and $CD8^+CD3^+$ are dark blue (top stacked bar).







T Cells expanded in PRIME-XV T cell Expansion XSFM maintain robust immunomodulatory and cytotoxic functionality after two weeks of expansion.

PBMCs were seeded in IL-7 (1000 IU/mL) + IL-15 (200 IU/mL) supplemented PRIME-XV T cell Expansion XSFM **(A)**, serum-containing medium **(E)**, and in leading suppliers' serum-free media **(B-D)** with anti-human CD3 and anti-human CD28 T cell activation beads in 24-well G-Rex culture vessels at a seeding density of 1 x 10^6 cells per well. Cells were harvested on day 14 of expansion and split into two groups. One group was stimulated with staphylococcus enterotoxin B (SEB), and the background group received PBS -/-. Following 2 hours of stimulation, cells were treated with a protein transport inhibitor cocktail, incubated for an additional 4 hours, and stained intracellularly for common functional markers. Cells were analyzed on the flow cytometer BD FACSymphony A3.

Related Products

Catalog #	Product	Size
500-01	CTGrade GMP rh IL-2$_{C126S}$	50 µg, 100 µg, 1 mg
500-07	CTGrade GMP rh IL-7	50 µg, 100 µg, 1 mg
500-08	CTGrade GMP rh IL-15	50 µg, 100 µg, 1 mg
500-04	CTGrade GMP rh IL-3	50 µg, 100 µg, 1 mg
500-05	CTGrade GMP rh IL-4	50 µg, 100 µg, 1 mg
91154	PRIME-XV T Cell CDM	1L bottle, 1L bag and 5L bag
91139	PRIME-XV FreezIS Solution	10 mL,100 mL
9160	RPMI Medium 1640 w/o L-Glutamine	100 mL, 500 mL
9161	RPMI Medium 1640 with L-Glutamine	100 mL, 500 mL
9236	PBS 1X-Dulbecco’s Phosphate Buffered Saline Solution	500 mL, 1 L
9240	PBS 1X-Dulbecco’s Phosphate Buffered Saline Solution	500 mL bottle, 1 L bottle, 1L bag, 5L bag

Technical Support

CONTACT US

For more information or assistance contact Customer Service at:

- Email: flbitmrequest@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)
- Certificate of Analysis (CoA) (when available)
- FAQs
- Product literature
- Complete list of offices and contact information by country

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