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T Cell Activation and Expansion in G-Rex Multi-Well Cell Culture Plates

The following protocol has been optimized for activation and expansion of T cells derived from Peripheral Blood Mononuclear Cells (PBMCs) with PRIME-XV T Cell CDM in G-Rex multi-well cell culture plate. Further optimization may be required depending on the desired application.

MATERIALS

- PRIME-XV T Cell CDM (cat. 91154, FUJIFILM Irvine Scientific)
- 1X sterile Dulbecco's Phosphate Buffered Saline (DPBS) without calcium and magnesium
- Anti-human CD3 antibody pure - functional grade (clone OKT3, cat. 130-093-387, Miltenyi Biotec)
- Ultra-LEAF Purified anti-human CD28 antibody (clone CD28.2, cat. 302933, BioLegend)
- Isolated PBMCs (fresh or frozen cells)
- Human recombinant IL-2 (rhIL-2, cat. 1420-050, CellGenix)
- G-Rex24 or G-Rex6 Well Plates (cat. 80192M or cat. 80240M, Wilson Wolf)
- Serological pipettes and sterile tips
- Disposable, sterile centrifuge tubes
- Conjugated antibodies for FACS analysis
- Lactate or glucose strips (optional)

INSTRUMENTS

- Pipettes and pipettors
- Biosafety cabinet
- Centrifuge
- 37°C, CO_2 incubator
- 37°C water bath
- Microscope
- Automated cell counter
- Flow cytometry (FACS)
- Lactate and/or glucose analyzers (optional)

EXPERIMENTAL PROCEDURE

Days 0-3: Seeding and T cell activation

1. Equilibrate sufficient amount of PRIME-XV T Cell CDM at 37°C for at least 15 minutes before using.
2. Prepare PBMC according to standard protocols (e.g. by Ficoll density gradient centrifugation) or rapidly thaw frozen cells at 37°C.
3. Transfer the cells into a centrifuge tube containing pre-warmed 1X DPBS (about 10 mL) for washing.
4. Determine viable cell concentration.
5. A small sample of the cell suspension could be analyzed by FACS to determine the initial phenotype (such as $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, $CD56^+$ and $CD14^+$).
6. Centrifuge cells at 300 × g for 5 minutes.
7. After the centrifugation, remove the supernatant.
8. Gently resuspend PBMC pellet in pre-warmed PRIME-XV T Cell CDM supplemented with 50 ng/mL soluble anti-CD3 monoclonal antibody (clone OKT3), 125 ng/mL soluble anti-CD28 monoclonal antibody (clone CD28.2) and 300 IU/mL IL-2.
9. Seed cell suspension at approximately 0.5×10^6 cells/cm^2 in the G-Rex Multi-Well Cell Culture Plate (about 1×10^6 cells/well in G-Rex 24-well plates and 5×10^6 cells/well in G-Rex 6-well plates) and add complete medium (supplemented with anti-CD3, anti-CD28 and IL-2), according to the manufacturer's instruction (e.g. 8 mL for G-Rex 24-well plates and 40 mL for G-Rex 6-well plates).

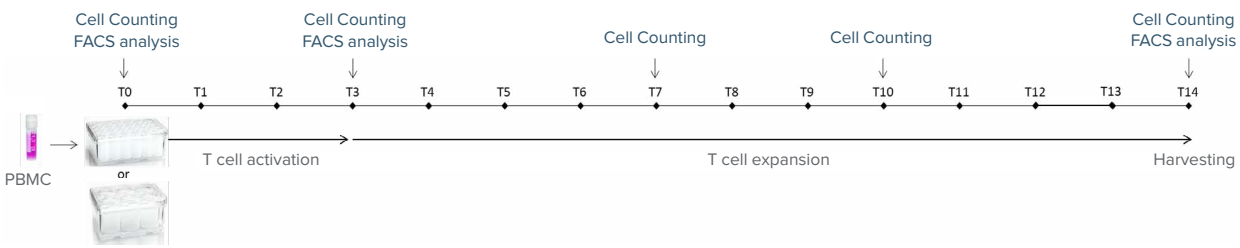

Figure 1. T Cell Activation and Expansion Workflow. PBMCs are seeded at 0.5 x 10^6 cells/cm^2 in G-Rex Well Plate, in PRIME-XV T Cell CDM supplemented with 300 IU/mL human recombinant IL-2 and soluble antibodies (50 ng/mL anti-CD3 and 125 ng/mL anti-CD28). On days 3, 7, 10, and 14, cell count and viability assessment are conducted according to Figure 1. Flow cytometry is performed on day 0, 3, and 14.

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EXPERIMENTAL PROCEDURE (CONTINUATION)

10. Incubate the G-Rex plate at 37°C in a humidified atmosphere with 5% CO_2.
11. After 72 h activation, gently remove 75% of medium volume from each well (about 6 mL for G-Rex 24-well plates and 30 mL for G-Rex 6-well plates) by pipetting from the top of the well without touching the bottom.
12. Resuspend the residual volume in the well (about 2 mL for G-Rex 24-well plates and 10 mL for G-Rex 6-well plates) and take a small sample for cell count.
13. Add fresh pre-warmed medium supplemented with IL-2 (300 IU/mL), according to the manufacturer's instruction, and place back into the incubator.
14. Glucose and lactate concentrations could be determined as proxy of the cell proliferation.
15. A small sample of cell suspension could be stained with markers of T cell activation (such as $CD25^+$ and $CD69^+$) and analyzed by FACS.

Days 7-10-14: T cell expansion

16. Gently remove 75% of medium volume/well, by pipetting from the top of the well down and resuspend the residual volume, taking a small aliquot for cell count.
17. Add pre-warmed medium supplemented with IL-2 (300 IU/mL) and place back into the incubator.
18. Glucose and lactate concentrations could be determined as proxy of the cell proliferation.
19. On Day 14, perform the count as described at point 16, harvest the cells, and process them for your assay of interest.
20. FACS analysis could be performed according to standard protocols (at least $CD3^+$, $CD4^+$, $CD8^+$).

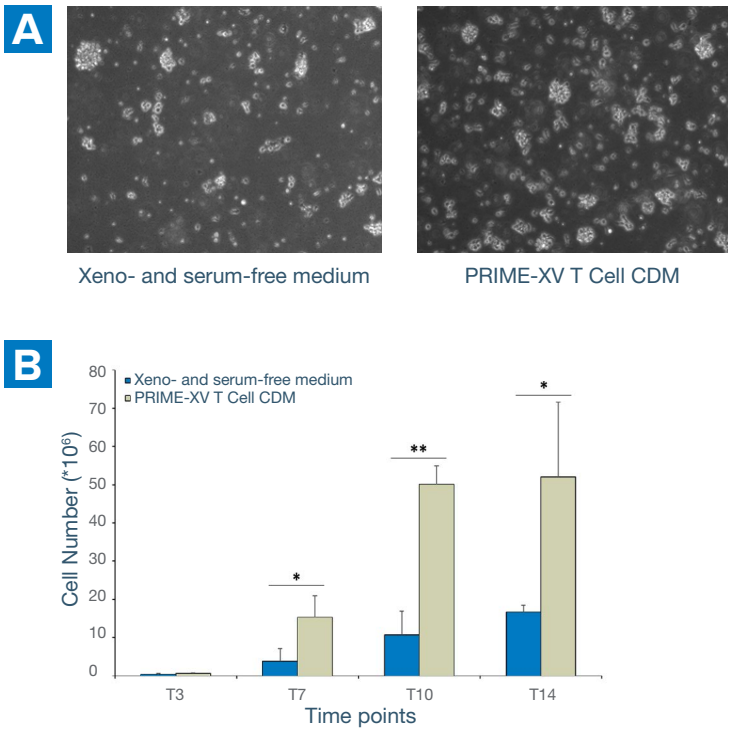

Figure 2. PRIME-XV T Cell CDM supports vigorous T cell expansion. T cells derived from human peripheral blood mononuclear cells, PBMC, of three healthy donors, were activated and cultured in G-Rex 24-well plates (0.5×10^6 cells/cm^2) in PRIME-XV T Cell CDM or in a commercially-available xeno- and serum-free medium. The media were supplemented with IL-2 and soluble antibodies (anti-CD3 and anti-CD28). On days 3, 7, 10, and 14, cell count and viability were assessed using Countess II (Thermo Fisher Scientific Inc.), and flow cytometry was performed using Sony SH800 (Sony).

(A) T cells exhibited an activated morphology (clusters) 72 hours after activation in the two tested media. Representative phase contrast images were taken on a Primovert microscope (Zeiss) (magnification 10x).

(B) Compared to a commercially-available xeno- and serum-free medium tested, PRIME-XV T Cell CDM medium supported a significantly higher expansion of total T cells. Each column with error bars represents the mean ± SD (n=3). * indicates $p \leq 0.05$, ** indicates $p \leq 0.01$; two-tailed Student's t-test was applied.

Protocol developed and owned by Anemocyte Srl.
Data provided courtesy of Anemocyte Srl.

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