PRIME-XV® NK CELL CDM

PRIME-XV® NK Cell CDM is an optimized chemically-defined media recommended for use in the expansion of human Natural Killer (NK) cells. The performance of this medium was assessed on NK cells derived from peripheral blood mononuclear cells (PBMC) and the immortalized NK cell line NK92. PRIME-XV NK Cell CDM is intended to be used with cytokine supplements for the ex vivo culture of NK cells. The cytokine cocktail used depends on the experimental requirements of each user.

Catalog #	Product	Size
91215	PRIME-XV NK Cell CDM	1 L Additional package sizes are available at request

Intended Use

For research or further manufacturing use only. Not for injection or diagnostic procedures.

Quality Assurance

All quality control test results are reported on a lot specific Certificate of Analysis (COA) which is available upon request.

Storage Instructions and Stability

Handle using aseptic techniques to avoid contamination. PRIME-XV NK Cell CDM should be stored at 2-8˚C and protected from light until ready to use. It is stable at 2-8˚C, under original packaging, for 1 year from date of manufacture. Once opened, the product can be stored at 2-8 in the dark and used within 4 weeks.

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 unopened expiry shelf life. Please refer to the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

Directions for Use

The following protocols are optimized for the expansion of PBMC derived NK cells or NK92 cells with PRIME-XV NK Cell CDM.

Protocol for PBMC NK derived Cell Expansion in the presence of feeder cells (K562)

Feeder cell inactivation

1. Equilibrate sufficient amount of PRIME-XV NK Cell CDM at 37˚C for at least 15 minutes before using.
   Note: To avoid temperature cycling, determine the total volume needed before equilibration.
2. Determine the amount of K562 cells needed for use at a ratio of 1:3 K562 cells to PBMCs. Example: for each $1x10^6$ PBMC cells, prepare $0.3x10^6$ K562 cells.
3. Spin K562 cells down at 300 g for 5 minutes.
4. Carefully aspirate supernatant leaving a minimum volume of media covering the cell pellet.
5. Resuspend K562 cell pellet in PRIME-XV NK Cell CDM at $0.5x10^6$ cells/mL and add mitomycin C at a final concentration of 20 mg/mL.
6. Incubate cells for 30 min in an incubator at 37˚C and 5% CO_2.
7. After the inactivation period, spin K562 cells down at 300 g for 5 minutes.
8. Carefully aspirate supernatant leaving a minimum volume of media covering the cell pellet.
9. Resuspend K562 cell pellet in PRIME-XV NK Cell CDM supplemented with 100 U/mL (5,500 IU/mL) of IL-2 at a cell density of $1x10^6$ cells/mL. This cell suspension is now ready to be directly added to the PBMC cell culture.

PBMC derived NK cell expansion

1. Equilibrate sufficient amount of PRIME-XV NK Cell CDM at 37˚C for at least 15 minutes before using.
   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.
3. Carefully transfer entire content of the vial into a 15 mL conical tube containing 10 mL of PRIME-XV NK 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 NK Cell CDM with 100 U/mL (5,500 IU/mL) of IL-2.
7. Resuspend cell pellet with the cytokine supplemented PRIME-XV NK Cell CDM and then transfer cells onto a plate at a density of $0.5x10^6$ cells/mL.
8. Add freshly inactivated K562 cells at a ratio of 1:3 K562 cells to PBMCs. For example, for each $1x10^6$ cells of PBMC cells, add 300 µL of inactivated K562 cell suspension.
9. Incubate cells in an incubator at 37˚C and 5% CO_2.

10. Feed cells with cytokine supplemented PRIME-XV NK Cell CDM every 2-3 days of culture, or when cells reach a density of 1-1.5x10^6 cells/mL, by spinning cells down at 300 g for 5 minutes and resuspend the pellet in fresh media at a density of 0.5x10^6 cells/mL.
11. Replenish feeder cells with freshly inactivated ones at day 7.

Protocol for NK92 Cell Expansion

1. Equilibrate sufficient amount of PRIME-XV NK Cell CDM at 37˚C for at least 15 minutes before using.
   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.
3. Carefully transfer entire content of the vial into a 15 mL conical tube containing 10 mL of PRIME-XV NK 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 NK Cell CDM with 10 U/mL (550 IU/mL) of IL-2.
7. Resuspend cell pellet with the cytokine supplemented PRIME-XV NK Cell CDM and then transfer cells onto a plate at 0.25x10^6 cells/mL.
8. Incubate cells in an incubator at 37˚C and 5% CO_2.
9. Feed cells with cytokine supplemented PRIME-XV NK Cell CDM every 2-3 days of culture by spinning cells down at 300 g for 5 minutes and resuspend the pellet in fresh media at 0.25x10^6 cells/mL.

Example Data

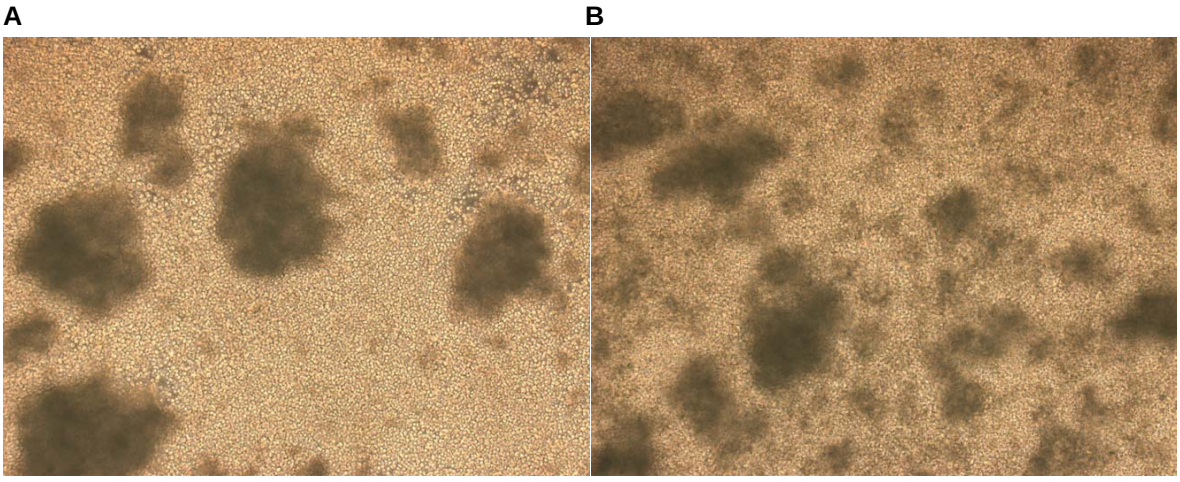

Figure 1. PRIME-XV NK Cell CDM maintains the characteristic morphology of PBMC-derived NK cells. PBMC cells were cultured for 14 days in PRIME-XV NK Cell CDM supplemented with 100 U/mL (5,500 IU/mL) of IL-2 without (A) or with feeder cells (K562) (B). The cells were plated at 0.5x10^6 cells/mL in tissue culture plastic plates. (10x magnification).

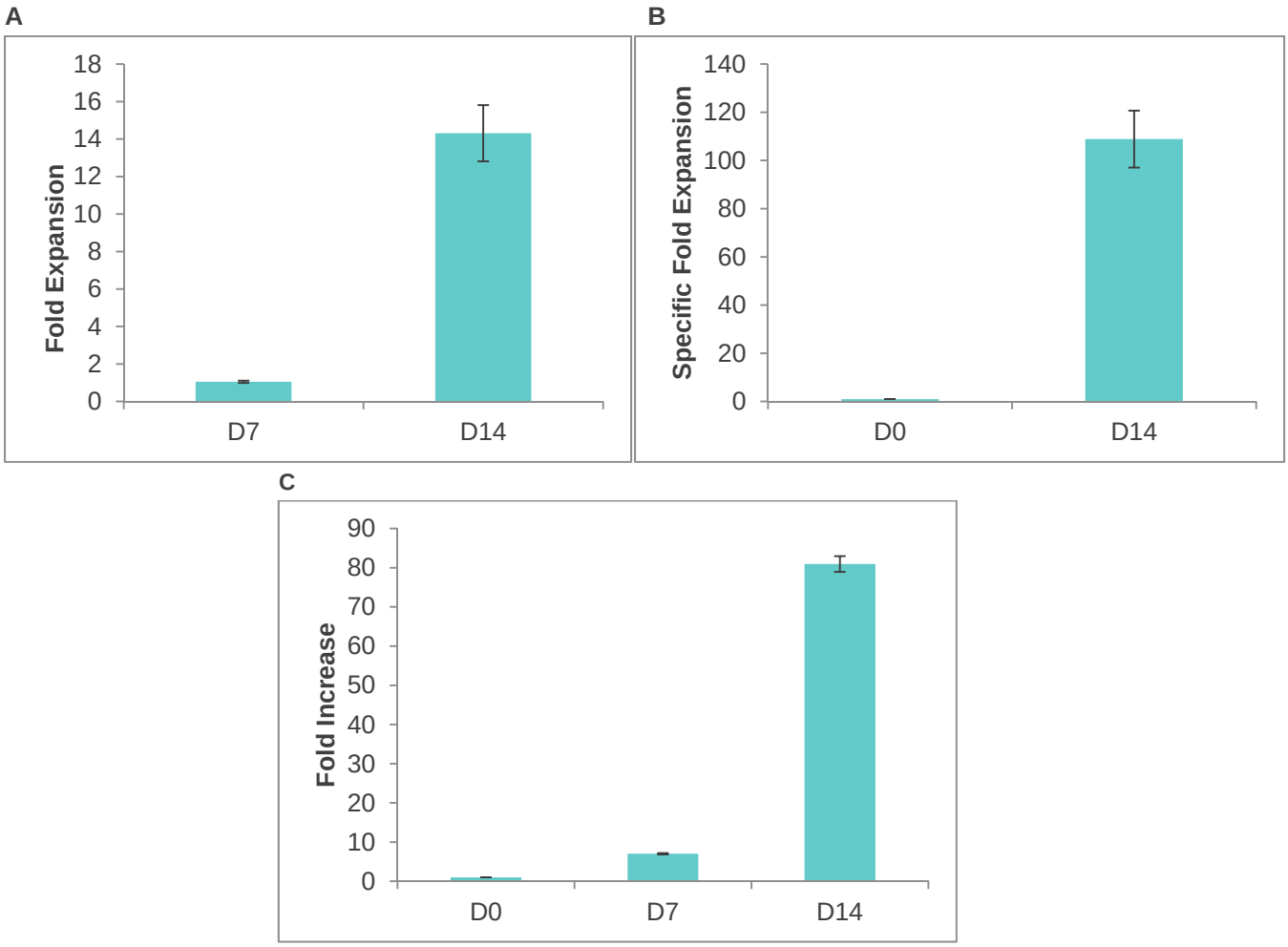

Figure 2. Expansion profile of PBMC-derived and immortalized NK cells in PRIME-XV NK Cell CDM. (A, B) PBMCs were cultured for 14 days in PRIME-XV NK Cell CDM supplemented with 100 U/mL (5,500 IU/mL) of IL-2. Feeder cells (inactivated K562) were added at day 0 and 7, at a ratio of 1:3 K562 to PBMCs. At day 7 and 14 fold increase (A) and specific fold increase (B) were quantified. Specific fold increase was measured as the fold increase in $CD3^{-}CD56^{+}$ cell population. (C) NK92 cells were cultured for 14 days in PRIME-XV NK Cell CDM supplemented with 10 U/mL (550 IU/mL) of IL-2. Fold increase was determined at day 7 and 14.

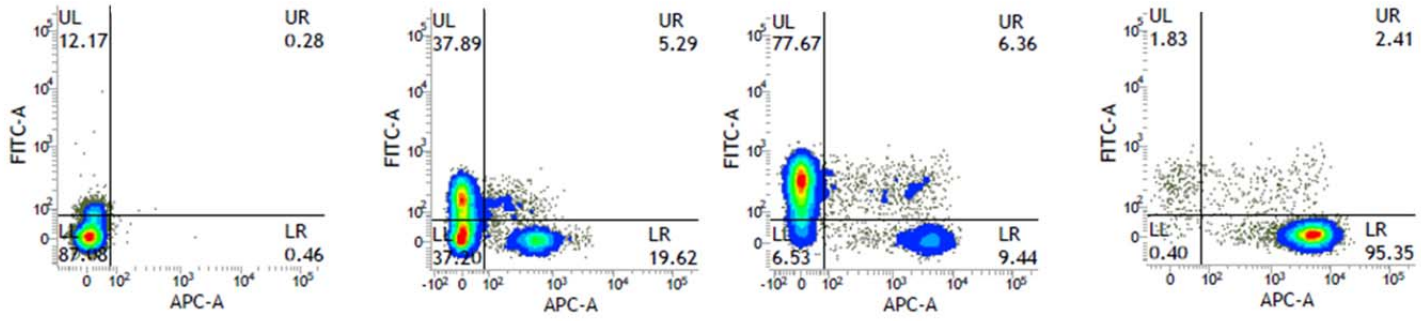

Figure 3. Flow Cytometry analysis of PBMC-derived NK cells in PRIME-XV NK Cell CDM. PBMC cells were cultured for up to 14 days in PRIME-XV NK Cell CDM supplemented with 100 U/mL (5,500 IU/mL) IL-2. Feeder cells were added at day 0 and 7. Flow cytometry analysis for CD3 (FITC-C) and CD56 (APC-A) was performed. Antibody-specific isotypes were used as negative controls (A), and cells were analyzed at day 0 (B), and at day 14 when grown without (C) or with K562 feeder cells (D).

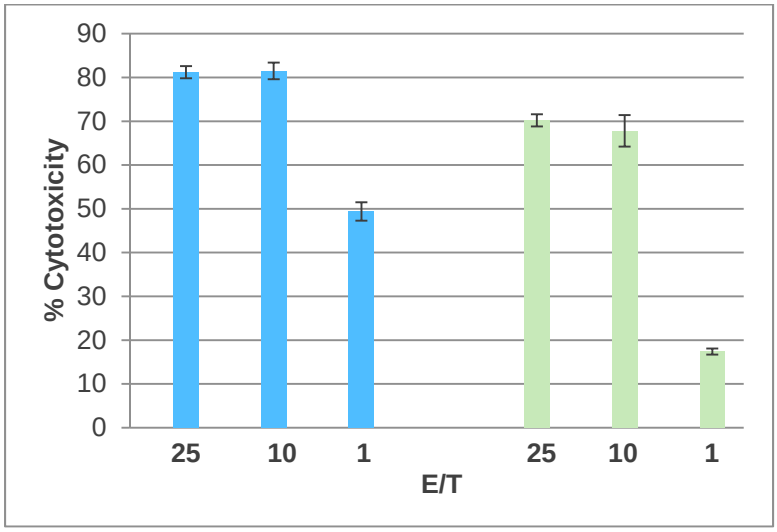

Figure 4. PRIME-XV NK Cell CDM supports expansion of PBMC-derived NK and NK92 cells with high cytotoxicity profile. PBMCs were cultured for 14 days in PRIME-XV NK Cell CDM supplemented with 100 U/mL (5,500 IU/mL) of IL-2. Feeder cells were provided at day 0 and 7. NK92 cells were cultured for 14 days in PRIME-XV NK Cell CDM supplemented with 10 U/mL (550 IU/mL) of IL-2. After 14 days in culture, cytotoxicity profiles were measured in a fluorescence-based assay against K562 cells, at 3 different effector: target ratios (25, 10 and 1). Blue = PBMC-derived NK cells; Green = NK92 cells

Related Products

Catalog #	Product	Size
9240	PBS, Dulbecco's Phosphate Buffered Saline	100 mL, 500 mL, 1L
95118	Recombinant Human IL-2 ACF	10 µg

Technical support

CONTACT US

For more information or assistance contact Customer Service at:

- Email: tmrequest@irvinesci.com
- Direct line: +1 800 577 6097

WEBSITE RESOURCES

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

- SDSs
- COAs (when available)
- FAQs
- Product literature
- Complete list of offices and contact information by country

Irvine Scientific
2511 Daimler Street, Santa Ana, California 92705-5588 USA
Telephone: 1 949 261 7800 • 1 800 437 5706
Fax: 1 949 261 6522 • www.irvinesci.com

 P/N 41104 Rev.0