PRIME-XV NK CELL CDM

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.

Product Description

PRIME-XV NK Cell CDM is an optimized chemically-defined media recommended for use in the expansion of human Natural Killer (NK) and NKT cells. The performance of this medium was assessed on NK cells derived from peripheral blood mononuclear cells (PBMC) and NKT. 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.

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 18 months from date of manufacture. Once opened, the product can be stored at 2-8°C 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 NK cells enriched from PBMCs and PBMC-derived NKT cells. with PRIME-XV NK Cell CDM.

PROTOCOL FOR PBMC-DERIVED NK CELL EXPANSION

Activation and expansion media preparation

1. Prepare 1 mL of seeding media (PRIME-XV NK Cell CDM) per $1x10^6$ enriched NK cells by adding the following recommended cytokine concentrations: 500 IU/mL recombinant human IL-2 (rhIL-2), 10 ng/mL rhIL-12, 10 ng/mL rhIL-18, and 10 ng/mL rhIL-21.
2. Prepare 50 mL of 2x expansion media (PRIME-XV NK Cell CDM) per $1x10^6$ enriched NK cells by doubling the relative concentrations of the cytokines presented in Step 1, as follows: 1000 IU/mL recombinant human IL-2 (rhIL-2), 20 ng/mL rhIL-12, 20 ng/mL rhIL-18, and 20 ng/mL rhIL-21.

NK cell preparation

3. 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.
4. Frozen cells: Thaw vial of cells by gently stirring in a 37˚C water bath for 1 minute.
   a. Carefully transfer entire contents of the vial into a 15mL conical tube containing 10 mL of PRIME-XV NK Cell CDM.
   b. Take a sample for counting. Spin cells down at 300 g for 5 minutes.
   c. Carefully aspirate supernatant leaving a minimum volume of media covering the cell pellet.
5. Fresh cells: Carefully transfer entire contents of the vial into a 15mL conical tube containing 10 mL of PRIME-XV NK Cell CDM.
   a. Take a sample for counting. Spin cells down at 300 g for 5 minutes.
   b. Carefully aspirate supernatant leaving a minimum volume of media covering the cell pellet.
6. If using whole PBMCs, enrich for NK cells using magnetic beads or a fluorescence activated cell sorter.
   a. Positive selection: select for $CD56^+$ cells.
   b. Negative selection: deplete $CD3^+$ cells.
   c. May use multiple enrichment methods, as necessary.
   d. Take a sample for counting. Spin cells down at 300 g for 5 minutes.
   e. Carefully aspirate supernatant leaving a minimum volume of media covering the cell pellet.
7. Phenotype cells using flow cytometry (Live/dead, CD3, CD56, CD16).
   a. If enriching NK cells, compare pre- and post-enrichment samples.

NK cell culture setup

8. Resuspend cell pellet in 1x seeding media (Step 1) at a concentration of 0.5×10^6 NK cells/mL
9. Activate cells as appropriate for application. FISI R&D used commercially available activation beads.

10. Aliquot 2 mL of the activated cell & cytokine mix into a T-25 flask and culture vertically in normal tissue culture conditions.
    a. 37˚C, 5% CO_2, humidified incubator.
    b. Incubate for up to 14 days.
11. Feed cells every 2-3 days by doubling the media volume with the 2x expansion media (Step 2).
    a. The culture volume may be transferred to larger vessels to accommodate the increase in volume over the two weeks.
    b. With every feed, assess the viable cell density, viability, and flow cytometry phenotype of the cells.
    c. Centrifugation of cells between feeds is unnecessary.

PROTOCOL FOR PBMC-DERIVED NKT CELL EXPANSION

NKT cell preparation

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. Frozen cells: Thaw vial of cells by gently stirring in a 37˚C water bath for 1 minute.
    a. Carefully transfer entire contents of the vial into a 15mL conical tube containing 10 mL of PRIME-XV NK Cell CDM.
    b. Take a sample for counting. Spin cells down at 300 g for 5 minutes.
    c. Carefully aspirate supernatant leaving a minimum volume of media covering the cell pellet.
3. Fresh cells: Carefully transfer entire contents of the vial into a 15mL conical tube containing 10 mL of PRIME-XV NK Cell CDM.
    a. Take a sample for counting. Spin cells down at 300 g for 5 minutes.
    b. Carefully aspirate supernatant leaving a minimum volume of media covering the cell pellet.
4. OPTIONAL: enrich for NK cells using magnetic beads or a fluorescence activated cell sorter
    a. Select for $CD3^+CD56^+$ cells.
    b. May use multiple enrichment methods, as necessary.
    c. Take a sample for counting. Spin cells down at 300 g for 5 minutes.
    d. Carefully aspirate supernatant leaving a minimum volume of media covering the cell pellet.
5. Phenotype cells using flow cytometry (Live/dead, CD3, CD56, CD16).
   If enriching NKT cells, compare pre- and post-enrichment samples.

NKT cell culture setup

6. Supplement appropriate volume PRIME-XV NK Cell CDM with 1000 IU/mL IL-2.
7. Resuspend NKT cell pellet in supplemented PRIME-XV NK Cell CDM at $0.5x10^6$ NKT cells/mL.
8. Aliquot 2 mL of the cell & cytokine mix into a T-25 flask and culture vertically in normal tissue culture conditions.
    a. 37˚C, 5% CO_2, humidified incubator.
    b. Incubate for up to 14 days.
9. Feed cells every 2-3 days by doubling the media volume using IL-2 supplemented PRIME-XV NK Cell CDM from Step 6.

a. The culture volume may be transferred to larger vessels to accommodate the increase in volume over the two weeks.
b. With every feed, assess the viable cell density, viability, and flow cytometry phenotype of the cells.
c. Centrifugation of cells between feeds is unnecessary.

Data

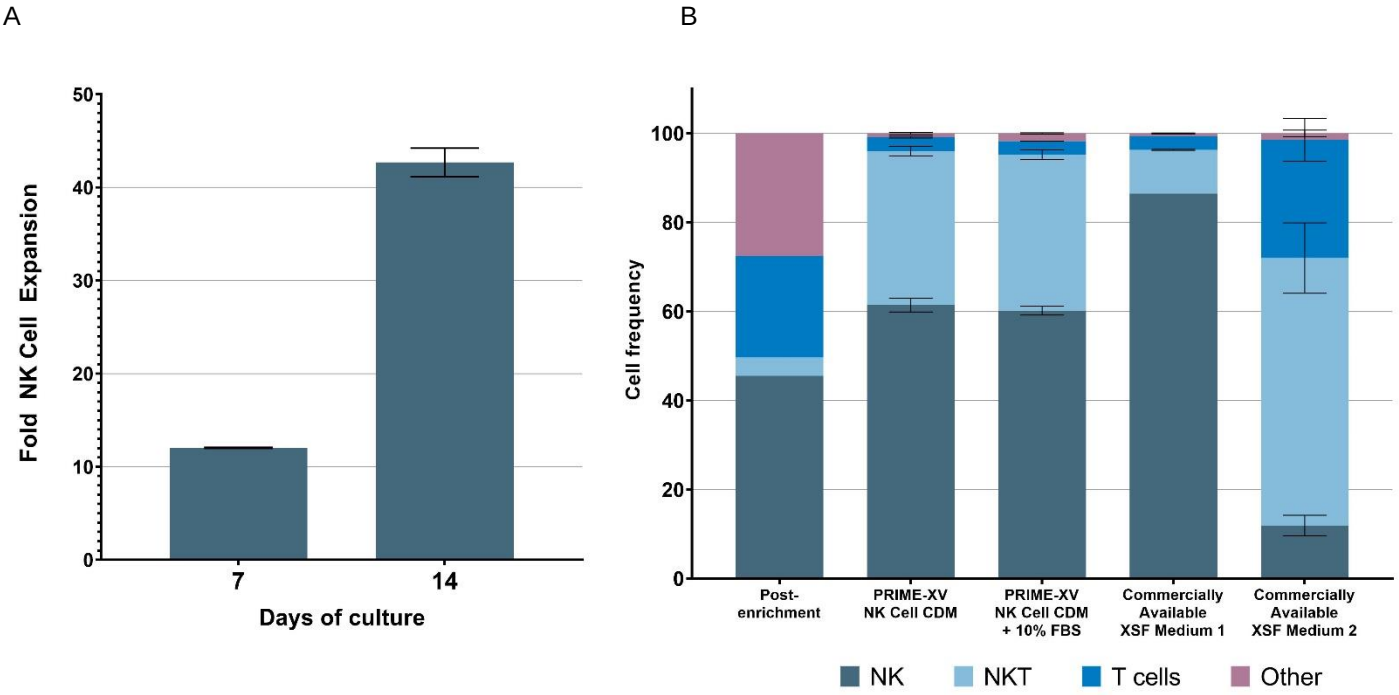

Figure 1. PRIME-XV NK Cell CDM preferentially expands NK and NKT cells in heterogeneous PBMC culture enriched for CD56⁺ cells. PBMC cells were enriched for CD56⁺ using magnetic cell sorting, activated using commercially available NK activation beads, and cultured for 14 days in PRIME-XV NK Cell CDM supplemented with 500 IU/mL rhIL-2, 10 ng/mL rhIL-12, 10 ng/mL rhIL-18, and 10 ng/mL rhIL-21. (A) Specific fold expansion of $CD56^{+}CD3^{-}$ cells on days 7 and 14 of culture. (B) Cell type composition at day 14 of culture.

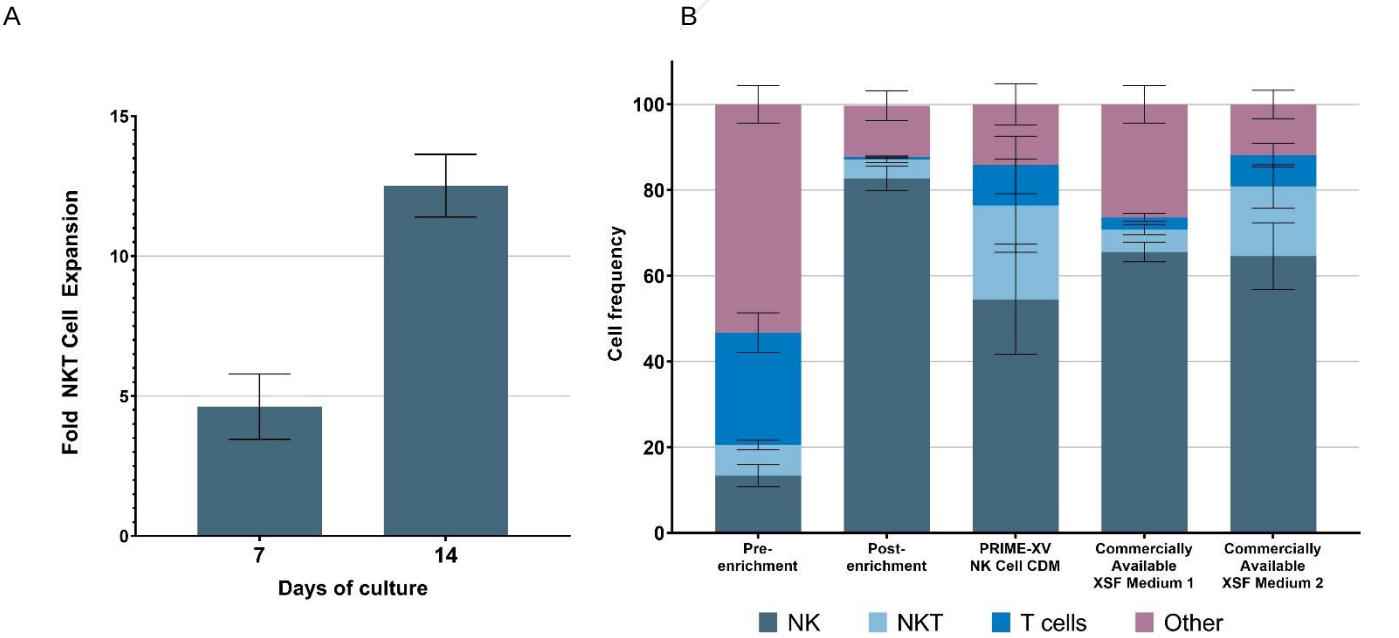

Figure 2. PRIME-XV NK Cell CDM supplemented with IL-2 expands NKT cells in PBMC culture enriched for CD56⁺ cells. PBMC cells were enriched for CD56⁺ using magnetic cell sorting and cultured for 14 days in PRIME-XV NK Cell CDM supplemented with 1000 IU/mL rhIL-2. (A) Specific fold expansion of $CD56^{+}CD3^{+}$ cells on days 7 and 14 of culture. (B) Cell type composition at day 14 of culture.

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: fisitmrequest@fujifilm.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

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