EXREnu Human Serum-Free NK Cell Expansion Kit

For Research Use Only. Not Intended for Diagnostic or Therapeutic Use.

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Catalog #	Product Name	Size	Components
EXCM005	EXREnu Human Serum-Free NK Cell Expansion Kit	3L/kit	• EXREnu NK Cell Stimulator 150 uL • EXREnu NK Cell Activator 500 uL x 3 • EXREnu NK Serum Free Base Medium 1L x 3
EXCM006		2L/kit	• EXREnu NK Cell Stimulator 100 uL • EXREnu NK Cell Activator 500 uL x 2 • EXREnu NK Serum Free Base Medium 1L x 2
EXCM007		1L/kit	• EXREnu NK Cell Stimulator 50 uL • EXREnu NK Cell Activator 500 uL • EXREnu NK Serum Free Base Medium 1L

Product Description

The EXREnu Human NK Cell Expansion Kit is optimized for high-density *in vitro* culture and expansion of human natural killer (NK) cells from human peripheral blood mononuclear cells (PBMCs). The EXREnu NK Serum Free Base Medium is serum-free and xeno-free to improve reproducibility and reduce risk of contaminating factors such as feeder cells and serum supplements utilized in earlier methods[1]. This chemically-defined medium contains albumin, transferrin, and insulin to support serum-free expansion of NK cells[2] with the addition of the optimized EXREnu NK Cell Activator and EXREnu NK Cell Stimulator included in the kit.

Intended Use

The EXREnu Human NK Cell Expansion Kit supports culture of NK Cells in a static culture vessel with medium at a recommended depth ≤ 1.5 cm to ensure good gas exchange. The kit also supports high-density culture of lymphocytes in a bioreactor, where conditions should be optimized by the requirements of each user. The addition of autologous plasma, human AB serum, FBS or serum substitutes can further improve the cell proliferation rate. Autologous plasma or Exreprotein™ Cell Culture Supplemental mix (Catalog #: EXCM008/EXCM009) is recommended to obtain the optimal culture results.

Shipping

- Base media shipped at 2-8°C. Upon receipt, store immediately at 2-8°C and **protect from light**.
- EXREnu NK Cell Stimulator and EXREnu NK Cell Activator shipped on dry ice. Upon receipt, store immediately at -20°C. Avoid repeated freeze-thaw cycles.

Storage

- Base media: 12 months from date of receipt at 2-8°C, **protected from light.**
- EXREnu NK Cell Stimulator and NK Cell Activator : 12 months from date of receipt at -20°C, **unopened**.
- EXREnu NK Cell Activator and EXREnu NK Stimulator vials should be used in their entirety, to avoid repeated freeze-thaw cycles.

Precautions

Aseptic techniques should be followed when handling the product and cells. Protective clothing should be worn, and safe laboratory procedures should be followed.

Limitations

- This medium is optimized to perform consistently. However, results may vary due to donor variability of primary cell populations and specific protocol design.
- EDTA plasma is not compatible. Please use heparin or sodium citrate plasma.
- Reduced cell viability and flocculation of cells may occur due to low temperatures of medium or if cell density is too high.
- Careful and gentle handling of the cells is recommended to avoid mechanical damage to cells.
- For Research Use Only. Not Intended for Diagnostic or Therapeutic Use.

Expansion of NK Cells

The following protocol describes the expansion of NK cells from human peripheral blood mononuclear cells (PBMCs). The protocol is for reference only and should be optimized based on the objectives of individual users.

1. Allow the EXREnu NK Serum-Free Base Medium to reach room temperature for proper equilibration. Ensure all subsequent steps are conducted under sterile conditions.
2. Prepare **Amplification Medium**: add 500uL of EXREnu NK Cell Activator per liter of EXREnu NK Serum-Free Base Medium. The Amplification Medium is stable for 3 weeks at at 2-8^{o}C, **protected from light.**
3. Using aseptic techniques, separate human peripheral blood mononuclear cells (PBMCs) from human peripheral blood using Exreprotein™ Human Lymphocyte Separation Tube (Catalog #: EXCM010/EXCM011), Exreprotein™ Human Lymphocyte Separation Medium (Catalog # EXCM012/EXCM013), or desired protocol.
4. Prepare **heat-inactivated autologous plasma**: obtain plasma by separation, heat-treat the plasma at 56^{o}C for 30 minutes, centrifuge the plasma at 1000xg for 10 minutes, and then obtain the heat-inactivated autologous plasma by collecting the supernatant.
5. On day zero, seed the peripheral blood mononuclear cells (PBMCs) obtained through separation into a T75 or T175 cell culture flask using the Amplification Medium (with 10 % heat-inactivated autologous plasma) at 2.5-3×10^6 cells/mL. Add the entire volume of EXREnu NK Cell Stimulator and place in a 5% CO_2 incubator at 37°C.
6. On day 3, add fresh Amplification Medium (with 10% heat-inactivated autologous plasma) at a final volume ratio of 1:2 (original medium to fresh Amplification Medium). For example, 10 mL of original medium is supplemented with 20 mL fresh Amplification Medium.
7. Starting on day 5, and continuing every 2 days, perform a cell count to determine the cell concentration. Supplement with fresh Amplification Medium (with 10 % heat-inactivated autologous plasma) to obtain a cell concentration of 0.8-1×10^6 cells/mL. (Cell density may be adjusted relative to the phenol red indicator in the medium. When cells are passaged, the cell density may be adjusted to 0.8-0.9×10^6 cells/mL if the media color is yellow and adjusted to 1×10^6 cells/mL if the medium color is red.)
8. On day 7, either transfer the culture to a larger bottle or transfer it into a cell culture bag, depending on the volume of the cell suspension. It's recommended to use Exreprotein™ Cell Culture Bag (Catalog #: EXCM015/EXCM016). Note that the maximum culture volume for a T75 flask is 40 mL and for a T175 flask is 200 mL. If the medium volume exceeds 200 mL, transfer the cell suspension into a cell culture bag.
9. After day 7, reduce the heat-inactivated autologous plasma to 1% in the Amplification Medium.
10. Proceed with cell proliferation and cell surface marker detection assays.

Data Examples

Fig A. CD3$^-$CD56$^+$ Cell Population

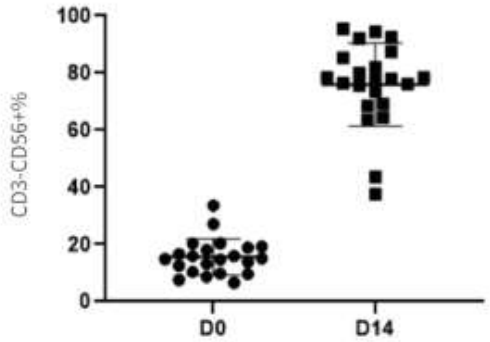

Fig B. CD3$^-$CD56$^+$ Cell Expansion

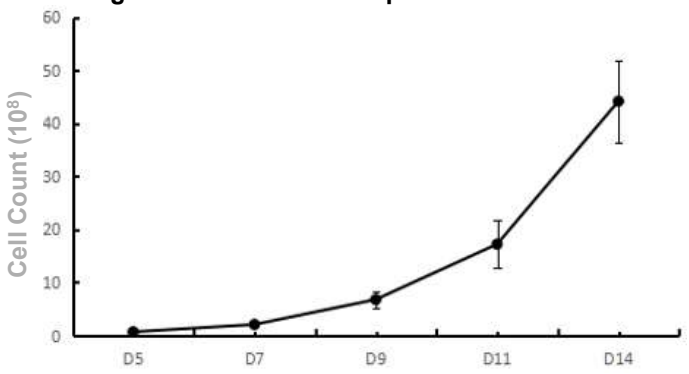

Fig A. After 14 days of culture, the population of CD3$^-$CD56$^+$ NK cells was between 60 to 95%.
Fig B. After 14 days of culture, 4 billion cells were harvested per 1L Amplification Media using the EXREnu Human NK Cell Expansion Kit. The typical expansion using this kit is 80 to 180-fold.

References

1. Robertson MJ, *et al*.(1996) Nat Immun. **15**:213.
2. Abel AM, *et al.* (2018) Front Immunol. **9**:1869.