

# EXREnu™ Human Serum-Free Lymphocyte Medium

Catalog Number: EXCM001

Size: 1000 mL

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

**EXREprotein™**

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## Product Description

EXREnu™ Human Serum-Free Lymphocyte Medium is optimized for high-density *in vitro* culture and expansion of human lymphocytes, cytokine-induced killer (CIK) cells, natural killer (NK) cells and dendritic cells from human peripheral blood mononuclear cells (PBMCs). This medium is serum-free and xeno-free to improve reproducibility and reduce risk of contaminating factors from earlier methods using feeder cells and serum supplements<sup>1</sup>. This chemically-defined medium contains albumin, transferrin, and insulin to support feeder cell-free and serum-free expansion of lymphocytes. Additional recombinant cytokines and activation methods are compatible with this medium for individually optimized growth and expansion of target lymphocyte populations.

## Intended Use

EXREnu™ Human Serum-Free Lymphocyte Medium supports culture of lymphocytes at a density of  $5 \times 10^5$  –  $5 \times 10^6$  cells/mL in a static culture vessel at a recommended depth  $\leq 1.5$  cm to ensure good gas exchange. The medium 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. The EXREnu™ Cell Culture Supplemental mix (Catalog #: EXCM008) is recommended to obtain the optimal culture result.

## Shipping

Shipped on blue ice. Upon receipt, store immediately at 2-8°C and **protect from light**.

## Storage

12 months from date of receipt at 2-8°C, **protected from light**.

## 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

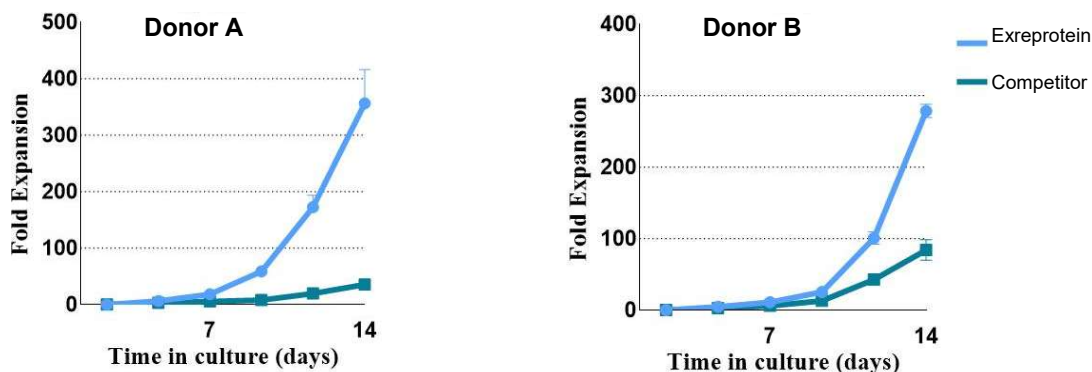
- This medium is optimized to perform consistently. However, results may vary due to donor variability of primary cell populations and specific protocol design.
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# Expansion of Cytokine-induced Killer (CIK) Cells

The following protocol describes the expansion of CIK cells from human PBMCs<sup>2</sup>. The protocol is for reference only and should be optimized based on the objectives of individual users.

1. Allow the EXREnu™ Human Serum-Free Lymphocyte Medium to reach room temperature for proper equilibration. Ensure all subsequent steps are conducted under sterile conditions.
2. Using aseptic techniques, separate human PBMCs from human peripheral blood using Exreprotein™ Human Lymphocyte Separation Tube (Catalog #: EXCM010/EXCM011), or desired protocol.
3. On day zero, seed the PBMCs obtained through separation into a T75 or T175 cell culture flask using the EXREnu™ Human Serum-Free Lymphocyte Medium at a concentration of  $2 - 2.5 \times 10^6$  cells/mL. Add recombinant human IFN-gamma (or use the optional EXREnu™ CIK Activation Kit, Catalog #: EXCM014), and place in a 5% CO<sub>2</sub> incubator at 37°C for 24 hours.
4. On day 1 (after 24 hours), stimulate the growth and proliferation of cytokine-induced killer cells by adding the appropriate activators and cytokines (CD3 agonist monoclonal antibody, recombinant human IL-2), according to the user-optimized protocol.
5. Starting on day 4, and continuing every 2 days, perform a cell count to determine the cell concentration. Supplement with fresh EXREnu Human Serum-Free Lymphocyte Medium (containing 1000 U/mL recombinant human IL-2) and adjust the cell concentration to  $1.5 \times 10^6$  cells/mL.
6. 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.
7. Proceed with cell proliferation and cell surface marker detection assays.

## Data Examples



After 14 days of serum-free culture, EXREnu™ Human Serum-Free Lymphocyte Medium showed 275 to 250-fold expansion of CD3<sup>+</sup> T cells and out-performed a similar medium from another supplier.

## References

1. Moore G.E., *et al.* (1967) JAMA. **199**:519.
2. Cappuzzello, E., *et al.* (2017) Cytokine Growth Factor Rev. **36**:99.