EXREnu™ Cell Culture Supplemental Mix

Catalog Number: EXCM008
Size: 250 mL or 25 mL

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

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Product Description

EXREnu™ Cell Culture Supplemental Mix is a serum substitute suitable for *in vitro* culture of human lymphocytes, cytotoxic T lymphocytes (CTLs), cytokine-induced killer (CIK) cells, natural killer (NK) cells and dendritic cells (DCs). This product contains growth factors, cytokines and proteins for cell expansion. It does not contain, nor use any raw materials that are derived directly from non-human animal sources during the manufacturing process. This product is optimized for lot-to-lot consistency.

Shipping

Shipped on dry ice. Upon receipt, store immediately at -15 to -25°C and **protect from light**.

Storage

36 months from date of receipt at -15 to -25°C, **protected from light. 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 product is optimized to perform consistently. However, results may vary due to donor variability of primary cell populations and specific protocol design.
- For Research Use Only. Not Intended for Diagnostic or Therapeutic Use.

Medium Preparation

1. Prepare 975 mL of serum-free medium. Allow it to reach room temperature.
2. Prepare 25 mL of Cell Culture Supplemental Mix and thaw in a water bath at 37°C, either centrifuge 3400 x g for 5 minutes to obtain supernatant or filter directly with a 40 µm cell strainer.
3. Add centrifuged supernatant or filtered Cell Culture Supplemental Mix to the serum-free medium.
4. The prepared complete medium can be stored at 2 to 8°C for 2 weeks, protected from light.
   Note: visible particles may form. This process is reversible when cells are placed in the incubator. It has no effect on cell viability, expansion, or differentiation.

Cytokine-induced Killer (CIK) Cells

The following protocol uses the EXREnu™ Cytokine-Induced Killer Cells Induction Culture Kit (Catalog # EXCM039).

1. On Day 0, seed the separated human peripheral blood mononuclear cells (PBMCs) into the culture flask ($2x10^6$ cells/mL, recommended density) using complete medium and add CIK Cytokine I at a final concentration of 1%, and culture in an incubator at 37°C, 5% CO_2.
2. On Day 1, add CIK Cytokine II at a final concentration of 1%.
3. On Day 3, supplement with an equal volume of fresh medium (containing 2.5% of Cell Culture Supplemental Mix and 0.05% CIK Cytokine III).
4. From Day 5, take samples for counting every other day, and fill up with fresh cell medium (containing 0.5% of this Cell Culture Supplemental Mix and 0.05% CIK Cytokine III) to maintain the cell density at $1x10^6$ cells/mL. From Day 7 (or when the culture volume is greater than 200 mL), select a larger flask or transfer into the cell culture bag according to the volume of cell culture suspension.

Natural Killer (NK) Cell Culture

The following protocol uses the EXREnu™ Human Serum-Free NK Cell Expansion Kit (Catalog # EXCM007).

1. On Day 0, add a vial of 500 µL NK Cell Activator to 1 L of EXREnu™ NK Serum-Free Base Medium to prepare the Amplification Medium.
2. Seed the separated human PBMCs into the culture flask ($2.5x10^6$ to $3.5x10^6$ cells/mL, recommended density) using the complete medium containing 10% Cell Culture Supplemental Mix (thawed in water bath at 37°C and filtered with a 40µm cell strainer), add NK Cell Stimulator, and place in incubator at 37°C , 5% CO_2.
3. On Day 3, supplement with fresh Amplification Medium (containing 5% Cell Culture Supplemental Mix) at a ratio of 1:1 of the original medium volume to fresh medium volume.
4. From Day 5, take samples every 2 days to count the cell concentration and supplement fresh Amplification Medium (containing 2.5% Cell Culture Supplemental Mix) according to the count results to adjust the cell concentration to $0.8x10^6$ to $1x10^6$ cells/mL. (The cell density can be adjusted approximately according to the color change of medium. During cell passage, if the medium is yellowish, the passage density can be adjusted to $0.8x10^6$ to $0.9x10^6$ cells/mL; if the medium is reddish, the passage density can be adjusted to $1x10^6$ cells/mL).
5. Around Day 7, use a larger flask or transfer into the cell culture bag (recommended use of EXREnu™ Cell Culture Bags, Catalog # EXCM015) according to the volume of cell culture suspension. The maximum culture volume of T75 flask is 40 mL and of T175 flask is 200 mL. When the medium volume exceeds 200 mL, transfer into the cell culture bag for culture.
6. The Cell Culture Supplemental Mix in the fresh Amplification Medium can be reduced to 1% after Day 7 and 0.5% after Day 11.