EXREnu™ Cytokine-Induced Killer Cells Induction Culture Kit

Catalog Number: EXCM039
Size: 1 Kit

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

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Product Description

The EXREnu™ Cytokine-Induced Killer (CIK) Cells Induction Culture Kit is optimized for *in vitro* expansion of human CIK cell culture. This product has stable batch-to-batch quality and can be used to expand CIK cells from human peripheral blood mononuclear cells (PBMCs).

EXREnu™ Cell Culture Supplemental Mix (Catalog # EXCM008) is recommended as an alternative to autologous plasma.

Shipping

- CIK Cytokines I, II and III shipped on dry ice. Upon receipt, store immediately at -15°C to -25°C.
- CIK Cell Serum-Free Medium shipped on blue ice. Upon receipt, store immediately 2°C to 8°C.

Storage

- CIK Cytokines I, II and III, store at -15°C to -25°C for 12 months, **avoid repeated freeze-thaw cycles**.
- CIK Cell Serum-Free Medium, store at 2°C to 8°C for 12 months, **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.
- Heparin is recommended as the anticoagulant for plasma collection. Do not use EDTA as an anticoagulant.
- Allow medium to reach room temperature prior to use.

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.

Materials Provided and Preparation

Reagents	Quantity
CIK Cytokine I	200 µL
CIK Cytokine II	200 µL
CIK Cytokine III	500 µL x 2
CIK Cell Serum-Free Medium	1 L x 2

Protocol

Heat-inactivated autologous plasma preparation: collect plasma using Heparin tubes and heat the plasma at 56°C for 30 minutes. Centrifuge at 1000 x g for 10 minutes and collect the supernatant to obtain heat-inactivated autologous plasma. EXREnu™ Cell Culture Supplemental Mix (Catalog # EXCM008) is recommended as an alternative to autologous plasma.

Sample requirements: use fresh or frozen human peripheral blood mononuclear cells (PBMCs) with viability ≥ 90%.

CIK Cell Culture (example using fresh PBMCs):

1. On Day 0, seed the separated PBMCs into the culture flask using CIK Cell Serum-Free Medium containing 5% heat-inactivated autologous plasma or 2.5% Cell Culture Supplemental Mix (seeding density recommended at 2 x 10^6 cells/mL) and add CIK Cytokine I at 1% of the medium volume. Culture in a 37℃ incubator containing 5% CO2.
2. On Day 1, add CIK Cytokine II at 1% of the medium volume.
3. Add 1 vial of 500 µL CIK Cytokine III (2000:1) to every 1 L of CIK Cell Serum-Free Medium to prepare the CIK Cell Expansion Medium. CIK Cell Expansion Medium can be stored at 4°C for up to 3 weeks.
4. On Day 3, fill up with an equal volume of fresh CIK Cell Expansion Medium (containing 5% heat-inactivated autologous plasma or 2.5% Cell Culture Supplemental Mix).
5. From Day 5, take samples for counting every other day, and fill up with fresh CIK Cell Expansion Medium (containing 1% heat-inactivated autologous plasma or 0.5% Cell Culture Supplemental Mix) to adjust the CIK cell density to 0.8-1.0 x 10^6 cells/mL. Select a larger flask or transfer into the cell culture bag 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.

Rapid Fluid Supplementation Procedure (3 x 10^7 PBMCs, for starting reference only):

Days	Original Volume (mL)	Supplementary Medium Volume (mL)	Supplementary Plasma Volume (mL)	Total Volume (mL)	Cytokine Added	Culture Flask/Bag
0	0	14.25	0.75	15	150 µL CIK Cytokine I	T75 culture flask
1	15	--	--	15	150 µL CIK Cytokine I	T75 culture flask
3	15	14.25	0.75	30	--	T75 culture flask
5	30	69.3	0.7	100	--	T175 culture flask
7	100	203.9	2.1	306	--	Culture bag
9	306	394	3.98	704	--	Culture bag
11	704	1232	--	1936	--	Culture bag

Notes:

- The recommended cell seeding density on Day 0 is 1.5-2.5 x 10^6 cells/mL for fresh PBMCs. For thawed PBMCs, it is recommended to increase the seeding density to 3 x 10^6 cells/mL.