DATA EXAMPLES

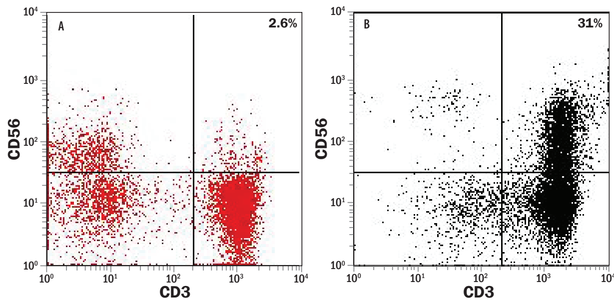
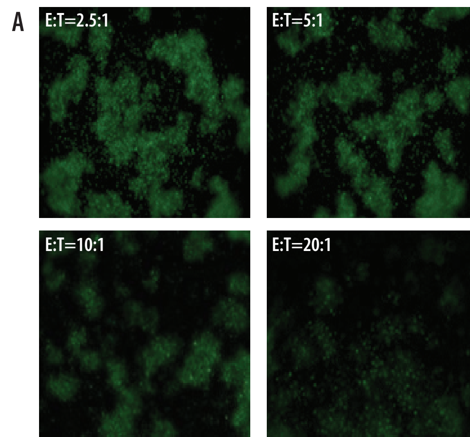


Figure 1: Flow Cytometry Analysis of Expanded Human CIK Cells. Human PBMCs were expanded *in vitro* for 21 days using reagents included in the CellXVivo™ Human CIK Cell Expansion Kit. Compared to unexpanded PBMCs **(A)**, PBMCs treated with the kit **(B)** show an increased number of $CD3^+CD56^+$ cells, which have the most potent cytotoxic function. Flow cytometric analysis was performed on day 21 of the expansion and cells were stained with Human NCAM-1/CD56 PE-conjugated Antibody and Human CD3ε PerCP-conjugated Antibody. Quadrants were set based on isotope-stained samples. All R&D Systems® antibodies and corresponding catalog numbers used are shown in the table below.

SUGGESTED REAGENTS FOR FLOW CYTOMETRY

CATALOG #	DESCRIPTION
FAB2408P	Human NCAM-1/CD56 PE-conjugated Antibody
FAB100C	Human CD3 epsilon PerCP-conjugated Antibody
IC0041P	Mouse IgG_{2B} PE-conjugated Antibody Isotype Control
IC002C	Mouse IgG_1 PerCP-conjugated Antibody Isotype Control

DATA EXAMPLES *CONTINUED*



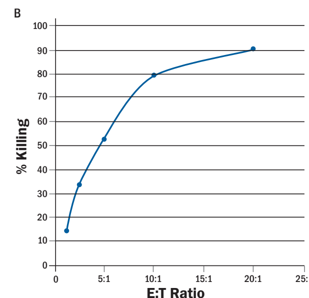


Figure 2: Kit-expanded CIK Cells are Cytotoxic to Tumor Cells. CIK cells expanded using the CellXVivo™ CIK Cell Expansion Kit were assessed for toxicity against the killer cell-sensitive K562 tumor cell line. K562 cell were loaded with the live-cell dye, Calcein-AM (Tocris®, Catalog # 5119) prior to being mixed with CIK cells for 4 hours at the indicated effector-to-target cell (E:T) ratios **(A)**. **(B)** Graph showing % killing of tumor cells by CIK cells at the indicated E:T ratios as determined by measuring relative fluorescence intensity of released Calcein-AM.

REFERENCES

1. Pittari, G. *et al.* (2015) Front. Immunol. **6**:230
2. Zhang, Q. *et al.* (2015) Hum. Immunol. **76**:3851
3. Guo, Y. and W. Han (2015) Chin. J. Cancer. **34**:99
4. Kim, J.S. *et al.* (2015) Immune Netw. **15**:58
5. Schmeel, F.C. *et al.* (2014) Int. J. Mol. Sci. **15**:14632

CellXVivo™

Human CIK Cell Expansion Kit

Catalog Number: CDK014

BACKGROUND

Cytokine-induced killer (CIK) cells exhibit phenotypic and functional similarities to both T cells and Natural Killer (NK) cells. While CIK cells express CD3 and expand readily in culture like T cells, they do not require functional priming for *in vivo* activity, a feature shared with NK cells (1). CIK cells consist of a heterogeneous population, including $CD3^+CD56^-$, $CD3^+CD56^+$, and $CD3^-CD56^+$ cells. Importantly, $CD3^+CD56^+$ have the most potent cytotoxic function among the CIK cells and express a number of cytokines, including IL-2, IFN-γ, TNF-α, and GM-CSF (2)(3). The advantages that CIK cells have over other routes of cell therapy include their ease of *in vitro* propagation and their ability to be primed without the need for exogenous administration of IL-2 (4). Over the past decade, cell therapy using CIK cells has emerged as an active area of research for the treatment of various types of cancer, such as hepatocellular carcinoma, non-small cell lung cancer, renal cell carcinoma, and gastric cancer (5). The CellXVivo™ Human CIK Cell Expansion Kit contains base media, recombinant human cytokines, a CD3 antibody, and a detailed protocol for the optimized expansion of highly cytotoxic $CD3^+CD56^+$ cells from peripheral blood mononuclear cells (PBMCs). This kit will expand a starting population of 25 x 10^6 PBMCs into 600 x 10^6 CIK cells.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at ≤ -20 °C in a manual defrost freezer. Do not use past kit expiration date.

COMPONENTS	PART #	# VIALS	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human Killer Cell Base Media 1	968074	500 mL	Store at 2-8 °C under sterile conditions for up to 1 month or at -20 °C to -70 °C in a manual defrost freezer for up to 3 months.*
Recombinant Human IFN-γ	968075	1 vial	
Recombinant Human IL-2	968076	1 vial	
Mouse Anti-Human CD3	968077	1 vial	
Reconstitution Buffer 1	967552	1 vial	Store at 2-8 °C under sterile conditions for up to 3 months.*
Reconstitution Buffer 2	967553	1 vial	

* Provided this is within the expiration date of the kit.

OTHER MATERIALS & SUPPLIES REQUIRED

- Ficoll-Hypaque™
- 75-cm² cell culture flask
- Microscope
- Hemocytometer
- 37 °C, 5% CO_2 humidified incubator
- Centrifuge
- Pipettes and pipette tips
- Sterile deionized water

REAGENT PREPARATION

Mouse Anti-Human CD3 (2000X) - Add 250 µL of Reconstitution Buffer 2 to Mouse Anti-Human CD3 stock solution to produce Mouse Anti-Human CD3 (2000X).

Recombinant Human IFN-γ (500X) - Add 100 µL of Reconstitution Buffer 1 to Recombinant Human IFN-γ to produce Recombinant Human IFN-γ (500X) stock solution.

Recombinant Human IL-2 (2000X) - Add 250 µL of Reconstitution Buffer 1 to Recombinant Human IL-2 to produce Recombinant Human IL-2 (2000X) stock solution.

Human Killer Cell Base Media 1 - Thaw Human Killer Cell Base Media 1 at 2-8 °C or at room temperature.

Human CIK Expansion Media 1 - Add Recombinant Human IFN-γ (500X) at a final concentration of 1X to the desired amount of Human Killer Cell Base Media 1. (e.g., for every 10 mL of base media, add 20 µL of Recombinant Human IFN-γ (500X)).

Human CIK Expansion Media 2 - Add Recombinant Human IL-2 (2000X) and Mouse Anti-Human CD3 (2000X) at a final concentration of 1X to the desired amount of Human Killer Cell Base Media 1. (e.g., for every 10 mL of base media, add 5 µL of Recombinant Human IL-2 (2000X) and Mouse Anti-Human CD3 (2000X)).

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PROTOCOL FOR CIK CELL EXPANSION

Note: *The following protocol is designed for a starting population of 25 x 10⁶ PBMCs in a single 75-cm² cell culture flask. For scale up, adjust volumes and flask quantity as needed.*

Seed Cells, Day 1

1. Isolate human peripheral blood mononuclear cells (PBMCs) from human blood using Ficoll-Hypaque density gradient centrifugation.
2. Prepare 30 mL of Human CIK Expansion Media 1.
3. Suspend isolated PBMCs in 5 mL of Human CIK Expansion Media 1.
4. Perform a cell count, and resuspend PBMCs at 1 X 10⁶/mL in Human CIK Expansion Media 1.
5. Transfer 25 mL of cell suspension into a 75-cm² flask, and incubate the flask in a 37 °C, 5% CO_2 humidified incubator.

Media Enhancement, Day 2

1. Add 12.5 µL of Mouse Anti-Human CD3 (2000X) and 12.5 µL of Recombinant Human IL-2 (2000X) into the cell culture in the 75-cm² flask.
2. Incubate the flask in a 37 °C, 5% CO_2 humidified incubator for 4 days.

Split Cells, Day 6

1. Prepare 50 mL of Human CIK Expansion Media 2.
2. Transfer cells from the 75-cm² flask into a 50 mL conical tube.
3. Centrifuge cells at 350 x g for 5 minutes.
4. Resuspend cell pellets in 50 mL of Human CIK Expansion Media 2.
5. Transfer 25 mL of suspended cells into each of two 75-cm² flasks.
6. Incubate the flasks in a 37 °C, 5% CO_2 humidified incubator for 6 days.

Split Cells, Day 12

1. Prepare 100 mL of Human CIK Expansion Media 2.
2. Transfer cells from each 75-cm² flask into a separate 50 mL conical tube.
3. Centrifuge cells at 350 x g for 5 minutes.
4. Resuspend each cell pellet in 50 mL of Human CIK Expansion Media 2.
5. Transfer 25 mL of suspended cells into each of four 75-cm² flasks.
6. Incubate the flasks in a 37 °C, 5% CO_2 humidified incubator for 6 days.

PROTOCOL FOR CIK CELL EXPANSION *CONTINUED*

Split Cells, Day 18

1. Prepare 200 mL of Human CIK Expansion Media 2.
2. Transfer cells from each 75-cm² flask into a separate 50 mL conical tube.
3. Centrifuge cells at 350 x g for 5 minutes.
4. Resuspend each cell pellet in 50 mL of Human CIK Expansion Media 2.
5. Transfer 25 mL of suspended cells into each of eight 75-cm² flasks.
6. Incubate the flasks in a 37 °C, 5% CO_2 humidified incubator for 6 days.

Harvest Cells, Day 21

1. Harvest expanded CIK cells.
2. CIK cells are ready for downstream applications.
3. **Optional:** To verify CIK cell expansion via flow cytometry, collect the cells and wash with PBS once. Process, stain, and analyze to determine CD3 and CD56 expression on the cell surface. Analyze marker expression via flow cytometry as shown in the Data Examples.

PROTOCOL OUTLINE