A New and Improved Kit for the Isolation of Untouched Human and Mouse NK Cells

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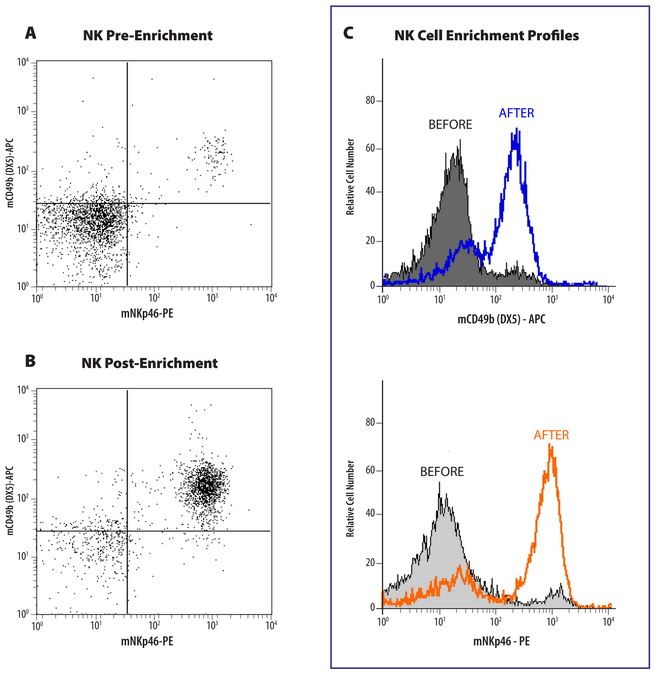
We have developed new MagCellect™ kits for the isolation of untouched natural killer (NK) cells from both mouse and human preparations. The new kits achieve levels of purity as high as 95%. Undesired populations are negatively depleted using a cocktail of monoclonal antibodies that specifically react with non-NK cell populations. These cell types are then tagged with magnetic beads and separated from the desired NK cells. A typical isolation is achieved in 45 minutes.

We have used the most recent and established markers and techniques to extensively characterize the isolated NK cells. We show here that the highly pure NK cell populations (both mouse and human) express NK cell-specific markers (i.e., NKp46, Nkp80, NKp30, CD56, NKG2D, KIR3DL1, and NTB-A in human, and NKp46, NKG2D, and CD49b in mouse samples). We also tested the functionality of the isolated NK cells. Isolated human NK cells were probed in a degranulation assay by measuring the expression of CD107a (LAMP-1) using flow cytometry after stimulation of isolated NK cells with myelogenous leukemia K562 cells.

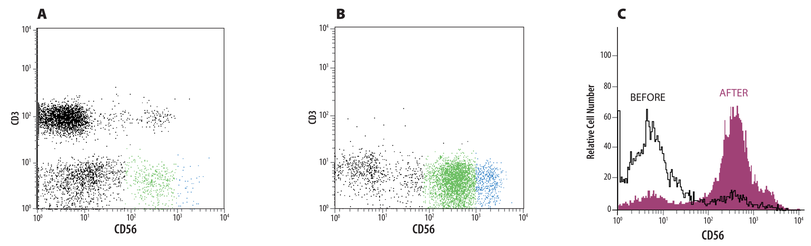
We compared the efficacy of our new MagCellect NK cell kits to other marketed systems, with typically better or similar results. In addition, unlike other commercially available kits that require brand-specific magnets and supplies, our kits were developed to work with most available magnets and with or without columns, providing more flexibility, simplicity, and cost-efficacy.

ISOLATION OF MOUSE NK CELLS



Example of enrichment of NK cells from mouse Balb/C splenocytes using the MagCellect NK cell enrichment kit (R&D Systems MAGM210). Cells before (panel A) and after (panel B) enrichment were double-stained with APC-conjugated anti-mouse CD49b (DX5) and PE-conjugated anti-mouse NKp46 (R&D Systems Catalog # FAB2225P) to specifically label the NK cell population. Two small populations with dim staining for DX5 and NKp46 were also enriched (panel C).

ISOLATION OF HUMAN NK CELLS

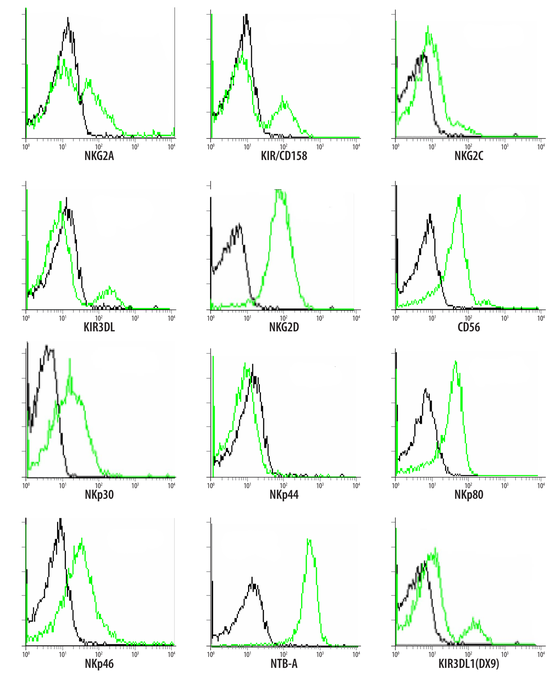


BEFORE ISOLATION	
CD56^{dim} (green)	8.5%
CD56^{bright} (blue)	0.9%

AFTER NK ISOLATION	
CD56^{dim} (green)	76.6%
CD56^{bright} (blue)	10.4%

Example of enrichment of NK cells from human PBMC using MagCellect NK cell enrichment kit (R&D Systems MAGH109). Cells before and after enrichment were double-stained with APC-conjugated anti-human CD3 (R&D Systems Catalog # FAB100A) and PE-conjugated anti-human CD56 (R&D Systems Catalog # FAB2408P). CD56^{dim} and CD56^{bright} NK cells are shown in green and blue respectively. The enrichment profile is also shown in a histogram.

PHENOTYPIC CHARACTERIZATION OF ISOLATED NK CELLS



List of R&D Systems APC- or PE-conjugated antibodies used in the phenotypic characterization of isolated NK cells:

ANTI-HUMAN	CATALOG #
NKp46	FAB1850A
NKp44	FAB22491A
NKp80	FAB1900A
NKp30	FAB1849P
CD56	FAB2408A
KIR3DL1	FAB12251A
KIR/CD158	FAB1848A
NTB-A	FAB19081A
NKG2D	FAB139A
NKG2A	FAB1059A
NKG2C	FAB138A

GENERAL METHODS

MOUSE SAMPLE PREPARATION:

A single cell suspension from Balb/C mouse spleen was resuspended in 1X MagCellect Plus buffer. Cell clumps and/or debris were removed by passing through a 40-70 µm nylon cell strainer. 50 million cells were used in a typical NK cell isolation.

HUMAN SAMPLE PREPARATION:

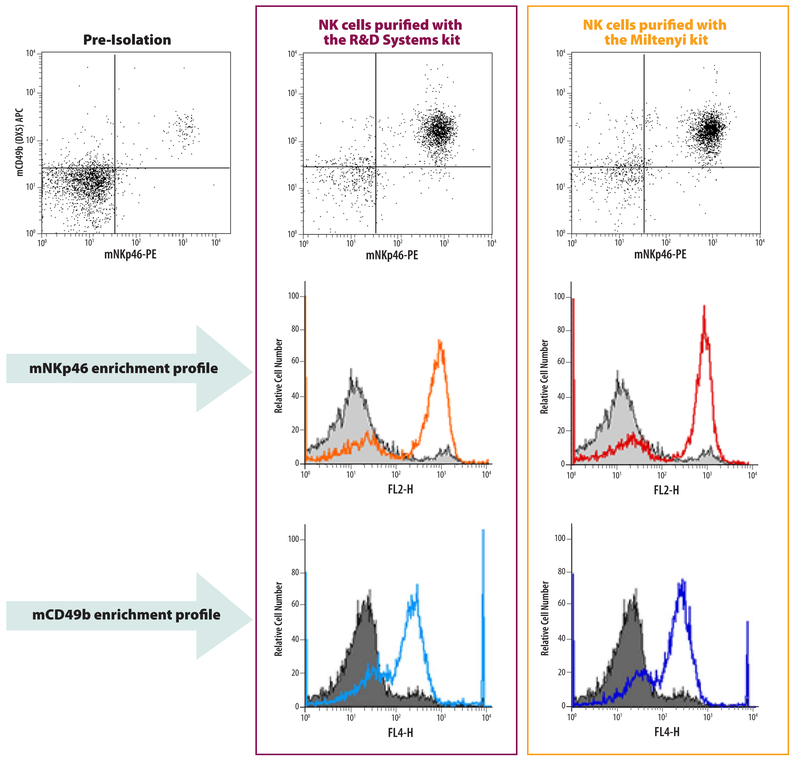
A single cell suspension of PBMC was prepared on a Ficoll Hypaque density gradient and resuspended in 1X MagCellect buffer. 50 million cells were used in a typical NK cell isolation.

ISOLATION OF NK CELLS

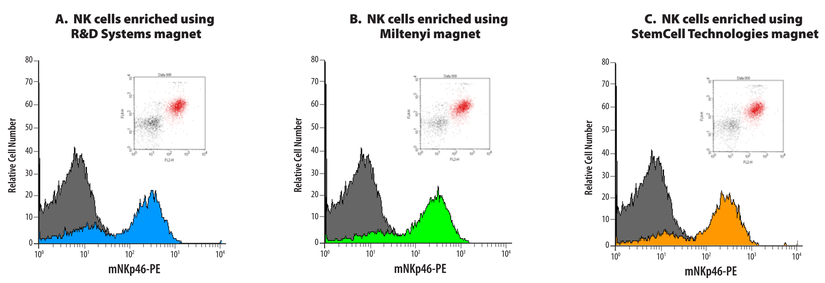
50 million mouse splenocytes or human PBMC were first incubated with MagCellect NK cell depletion cocktail for 15 minutes at 4°C, followed by the addition of streptavidin-conjugated magnetic ferrofluid for another 15 minutes. Cells were subsequently placed in a magnet for 6 minutes and NK cells in the supernatant were collected. Isolated cells were characterized and their functionality tested.

All other methods are detailed with the data.

COMPARING MOUSE MAGCELLECT KIT TO OTHER AVAILABLE KITS



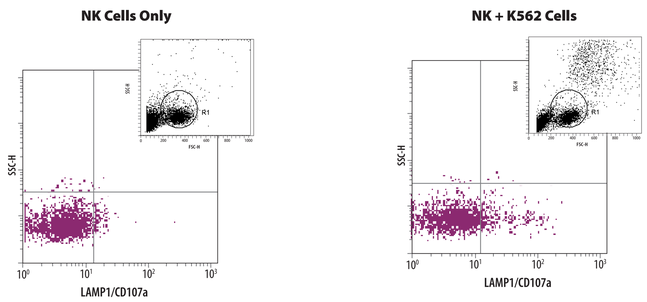
USING MOUSE MAGCELLECT (MAGM210) IN COMBINATION WITH COMPETITORS' MAGNETS AND COLUMNS



Enrichment of NK cells from Balb/C mice using R&D Systems MAGM210 in combination with either A) R&D Systems magnet (Catalog # MAG997), B) Miltenyi Biotec magnet (Catalog # 130-042-302) and LS columns (Catalog # 130-042-401) or C) StemCell Technologies magnet (Catalog # 18000).

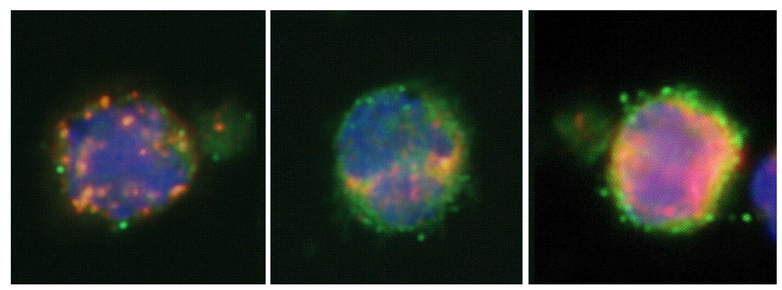
ASSAYS TO TEST THE FUNCTIONALITY OF ISOLATED NK CELLS

NK CELL DEGRANULATION ASSAY



Enriched human NK cells were functional as demonstrated with a CD107a degranulation potential assay. NK cells were incubated with K562 target cells at an effector/target (E/T) ratio of 2:1 for 3 hours (Ravet, S. *et al* (2007) Blood **109**:4296). Cells were then stained with APC-conjugated anti-human CD107a/LAMP-1 (R&D Systems Catalog # IC4800A) and analyzed by flow cytometry.

GRANZYME EXPRESSION IN ISOLATED NK CELLS



Cells were stained with anti-human NKp46 (Catalog # AF1850) followed by NorthernLights™493-conjugated anti-goat IgG (Catalog # NL003; green), and with anti-human Granzyme B (Catalog # MAB2906) followed by NorthenLights 557-conjugated anti-mouse IgG (Catalog # NL007; red). Nuclei were stained with DAPI (blue).

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