FUNCTIONAL HUMAN NK CELLS ISOLATED BY DYNABEADS® FLOWCOMP™ HUMAN NKp46

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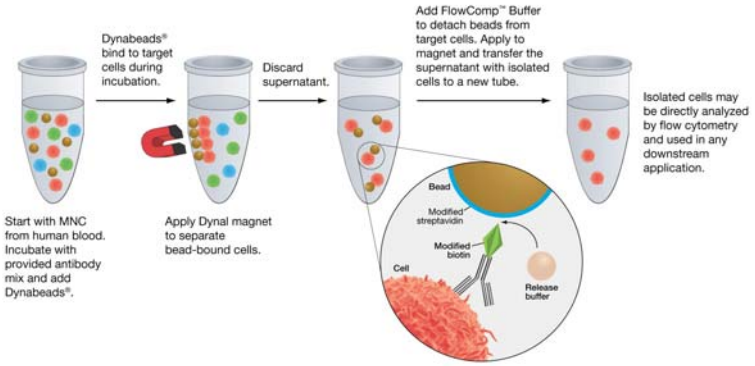
BACKGROUND: NK cells contribute to a variety of innate immune responses to viruses, tumors and allogenic cells. The potential of NK cells to be exploited in e.g. cancer immunotherapy have encouraged the development of new and exciting research areas. NK cell research is today one of the most expanding and promising areas in the therapeutic immunology field. In humans NK cells usually express CD56, however, this surface marker is not exclusive for NK cells and separation technology focusing on this marker will potentially results in contamination of non-NK cells. In contrast, the natural cytotoxicity receptor NKp46 is selectively expressed on all peripheral blood NK cells in healthy individuals and is a superior pan NK cell specific marker conserved between human, mouse and also other mammalian species.

MATERIALS AND METHODS: Human NKp46$^+$ cells were isolated using **Dynabeads® FlowComp™ Human NKp46 (cat.no. 113.64D)**. Peripheral blood mononuclear cells (PBMC) were isolated from healthy individuals. NKp46-specific antibody conjugated to a modified biotin was added and incubated at 2-8°C. In the next step, modified streptavidin coated Dynabeads were added and placed in the magnet after incubation at room temperature. After resuspension with FlowComp™ Release Buffer the bead-free cells were analyzed by flow cytometry and tested in cytotoxicity assays. LAMP-1 expression was assayed by flow cytometry after 4h incubation with target cells, and cytotoxicity of MHC class I deficient target cells was measured in a ^{51}Cr release assay.

Single Step Positive Isolation Procedure



AIM: Develop a single step isolation protocol for human pan-NK cells

RESULTS:
Highly pure human NKp46$^+$CD56$^+$CD3$^-$ NK cells were isolated from healthy blood donors using **Dynabeads® FlowComp™ Human NKp46** (fig. 2). NKp46 is a specific pan-NK cell marker and the isolated cells showed normal distribution of CD56^{hi}, CD56^{dim}, CD8$^+$ and CD8$^-$ subsets (fig. 3) and expression of KIR receptors (fig. 4). Positive isolation by NKp46 represents a unique product and results in high recovery of highly pure NK cells in a single positive isolation step (fig. 5). Only few CD3$^+$ T/NKT cells were present after isolation (fig. 2, 3). However, if higher purity is required, Dynabeads® CD3 (cat.no. 111.51D) can be used to further deplete CD3$^+$ cells. Isolated NK cells were 95% (SD 2.2) viable after isolation (fig. 6). Viability (propidium iodide negative cells) was measured on total events and represents the viability of the total yield. In contrast, when isolating NK cells with a column-based protocol, the viability was as low as 79% (SD 18.2) (fig. 6). Isolated NK cells were used in functional assays where LAMP-1 expression was assayed by flow cytometry (fig. 7) and where cytotoxicity was measured in a ^{51}Cr release assay using K562 as target cells (fig. 8). We demonstrate that isolated NK cells were activated and efficiently kill target cells.

KIR expression on isolated NK cells

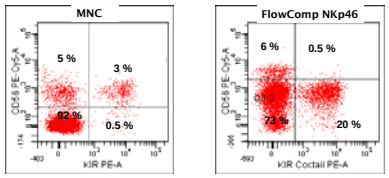


Figure 4. KIR expression on cells from MNC sample and from isolated NK cells. KIR expression was demonstrated by a PE-labeled CD158a and CD158b cocktail.

High yield and purity of NKp46$^+$ NK cells using Dynabeads®

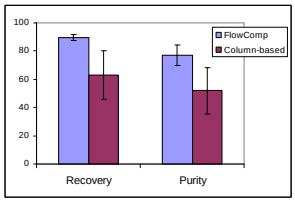


Figure 5. NK cells isolated with Dynabeads® FlowComp Human NKp46 yields higher recovery and purity of NK cells compared with column-based technology (anti-CD56). NK cells were identified as NKp46$^+$CD3$^-$ cells by flow analysis.

Higher viability of NKp46$^+$ NK cells using Dynabeads® compared to column-based method

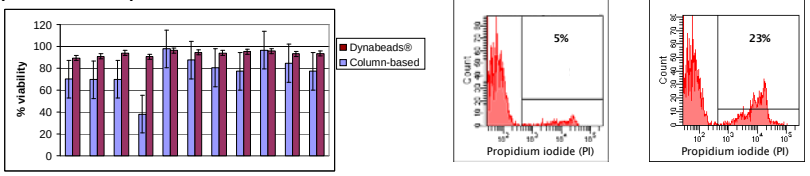


Figure 6. NK cells were isolated from 11 different donors using Dynabeads® FlowComp™ Human NKp46 or by column-based method, and analyzed for viable cells. Viable cells were identified by propidium iodide (PI) negative cells among all events. Importantly, viability of isolated cells varied very little between donors when using Dynabeads® FlowComp™ Human NKp46 (95% viability, STD 2.2), whereas a column-based method resulted in large variation between experiments (79% viability, STD 18.2).

Presence and percentage of NKp46$^+$ cells in PBMC and isolated cells

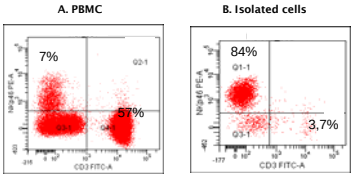


Figure 2. Isolation of NK cells with Dynabeads® FlowComp™ Human NKp46 **A)** In PBMC, about 8 % of the cells are NKp46$^+$. **B)** The isolated NKp46$^+$ cells are >80% pure. Contamination by CD3$^+$ NKT/T cells is minimal.

NK phenotype after isolation

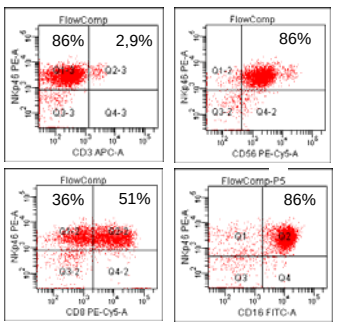


Figure 3. Phenotype of NK cells isolated by Dynabeads® FlowComp™ Human NKp46. NKp46 is a pan NK cell marker and all subsets of NK cells are present within the isolated population.

LAMP-1 expression on activated NKp46$^+$ NK cells

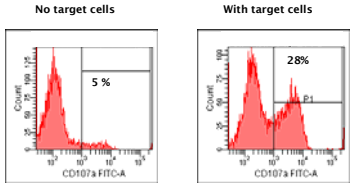


Figure 7. NK cells degranulate when stimulated with K562 target cells at E/T ratio 2:1 as measured by LAMP-1 (CD107a) expression.

Capability of NKp46$^+$ NK cells to kill target cells after Dynabeads® FlowComp™ isolation

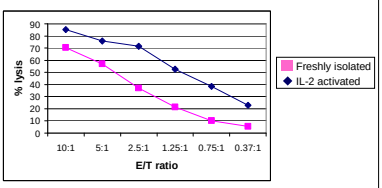


Figure 8. Freshly isolated and IL-2 activated NK cells efficiently kill MHC class I deficient target cells (K562) in a 4h ^{51}Cr release assay.

CONCLUSIONS

- **Dynabeads® FlowComp™ Human NKp46** is a unique product for isolation of highly pure NK cells in a single step protocol.
- Isolated NK cells are ≥ 80% pure NKp46$^+$CD56$^+$CD3$^-$ cells.
- NKp46$^+$ isolated cells express the expected CD56^{hi} CD56^{dim}, CD8$^+$ and CD8$^-$ subsets.
- NK cells isolated by Dynabeads® FlowComp™ technology are bead-free and ready for flow cytometric analysis and further downstream applications.
- Dynabeads® FlowComp™ technology yields more viable cells after isolation than when using column-based methods.
- Isolated NK cells efficiently kill target cells in cytotoxicity assays.

Ordering information	Cat.no	Application
Dynabeads® FlowComp™ Human NKp46	113.64D	Positive pan-NK cell isolation
Dynabeads® CD3	111.51D	Depletion of residual CD3$^+$ cells