



Miltenyi Biotec

# Clinical-grade NK cell manufacturing

Efficient cell separation, engineering,  
and expansion for the next front in cell therapy



# Your strategy. Our solutions. Unleash the power of NK cells.



## NK cells enhance the cell therapy armory

NK cells are the first line of defense against viruses and malignant cells, preventing tumor growth and dissemination.<sup>1,2</sup> They eliminate target cells via different mechanisms, including direct release of cytotoxic granules and antibody-mediated cellular cytotoxicity (ADCC).<sup>3</sup> Researchers have leveraged these unique defense functions in allogeneic adoptive cell therapies<sup>4,5</sup> and aim to enhance their anti-tumor effect in several immunotherapy approaches.

The importance of NK cells for novel cell and gene therapy (CGT) strategies is undeniable. Miltenyi Biotec solutions help scientists unleash the power of NK cells by eliminating bottlenecks in their manufacture

## Efficient solutions for NK cell manufacturing

Different processes on the CliniMACS® Plus and CliniMACS Prodigy® Instruments enable a range of strategies to generate GMP-grade, highly viable, and functional NK cells. Both platforms enclose and automate the efficient depletion of T cells and/or enrichment of NK cells. The final NK cell-enriched products are then ready for either direct use in adoptive therapies or for downstream manufacturing steps (fig. 1).

Designed as a versatile, closed, and integrated system, the CliniMACS Prodigy Instrument simplifies further steps in manufacturing native and genetically modified NK cells. It automates cell expansion and viral transduction (optional) in a standardized and GMP-compliant workflow (fig 2). Primary NK cells are efficiently transduced using baboon envelope pseudotype lentiviral vectors (BaEV LVV).<sup>6</sup> With our longstanding expertise in lentiviral vector design and production, including BaEV LVV, manufacturing on the CliniMACS Prodigy Platform promotes the successful generation of gene-engineered NK cells such as chimeric antigen receptor (CAR) NK cells.



Simplify and standardize your workflow for CAR NK cell manufacturing.

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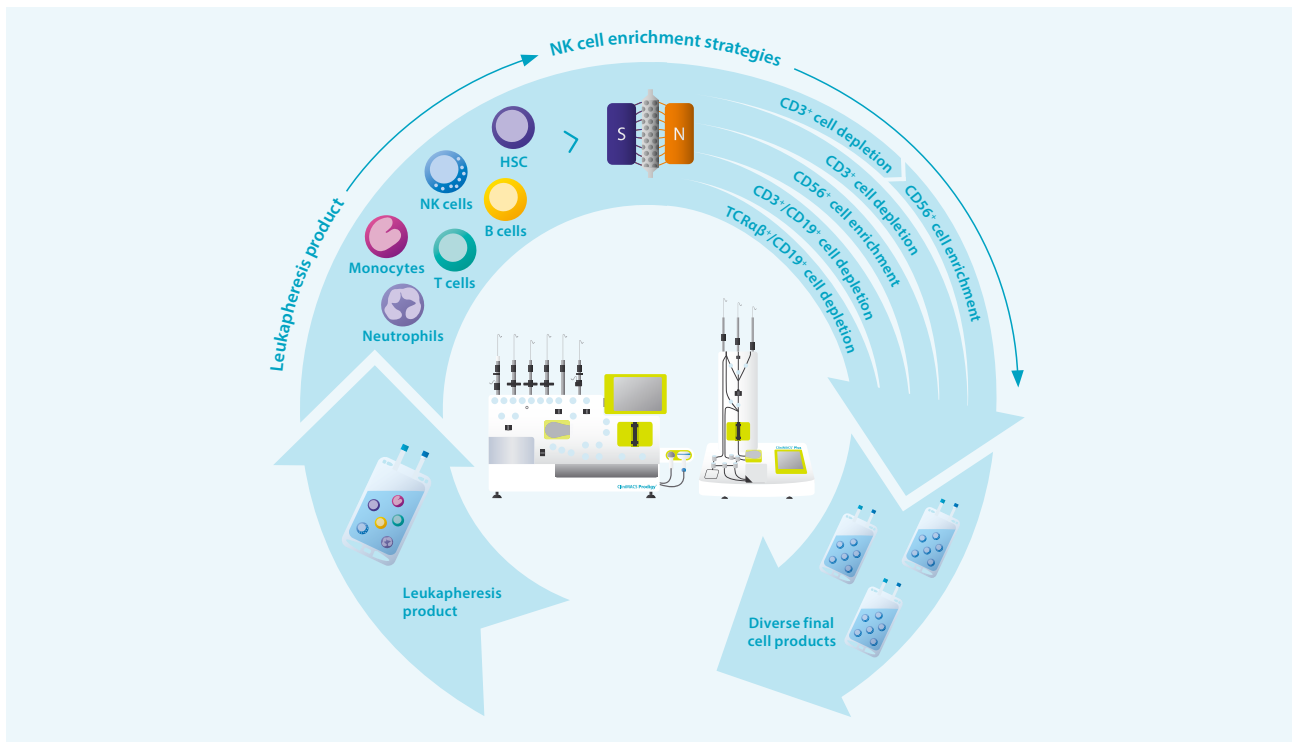


Get expert help in tailored lentiviral vector design and production to engineer NK cell.

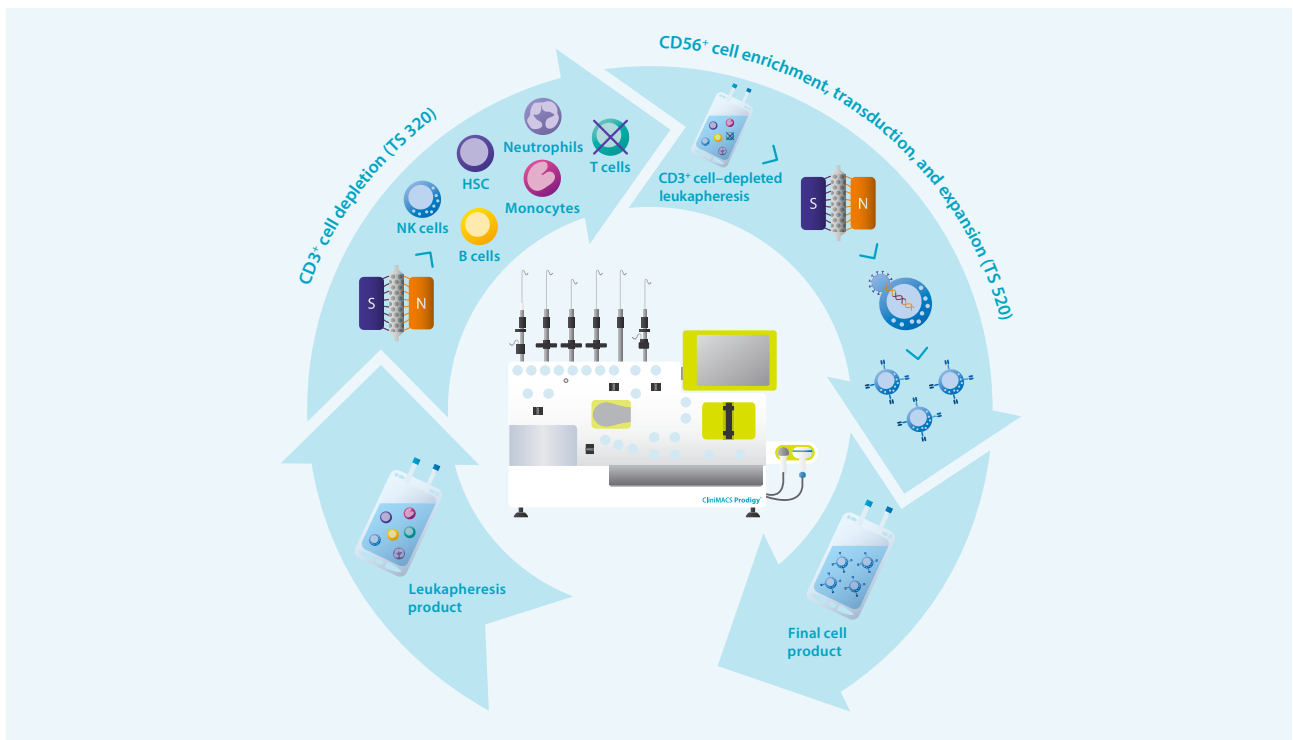
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**Figure 1: Various process options to generate different NK cell-enriched products.** The CliniMACS Prodigy and the CliniMACS Plus Instruments automate the separation of NK cells via different strategies.



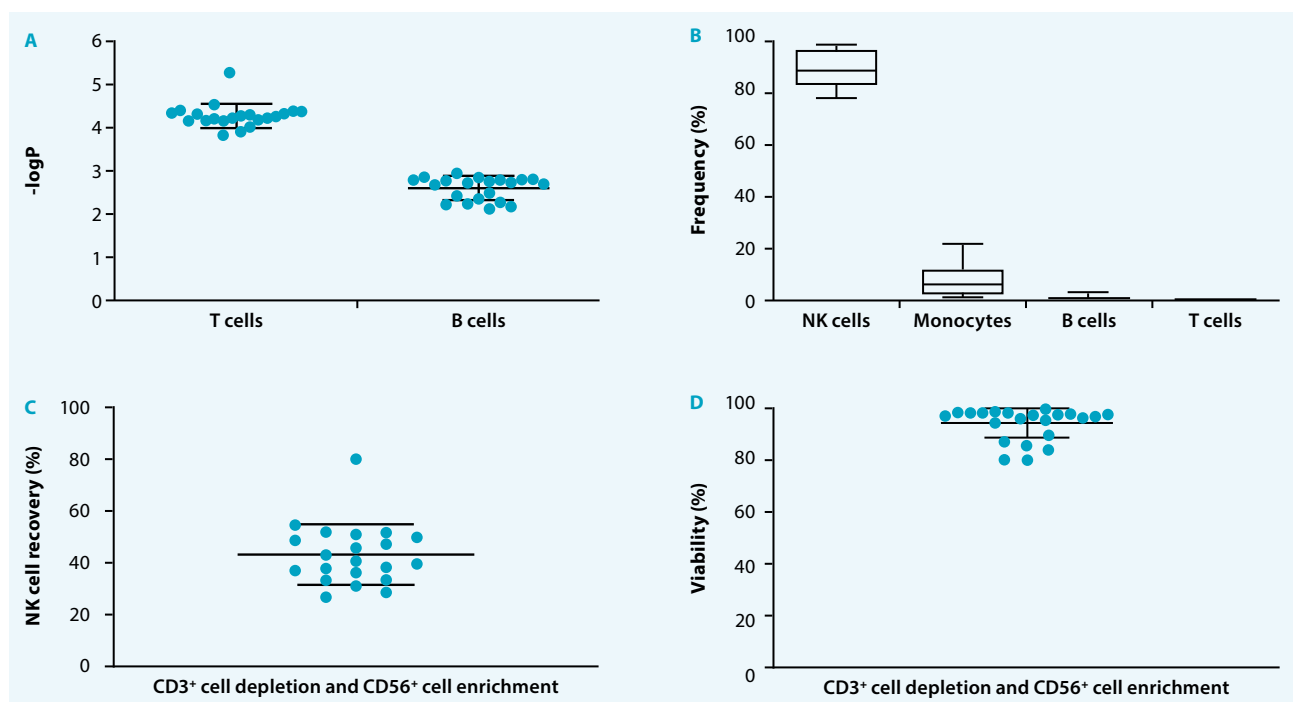
**Figure 2: The complete workflow for NK cell manufacturing.** In addition to cell separation, the CliniMACS Prodigy Instrument automates the activation, transduction, and expansion of NK cells.

# A pure start for immunotherapy

## NK cells in your product. And little else.

Over the years, Miltenyi Biotec has perfected clinical-grade magnetic cell separation. Automated on the CliniMACS Plus or the CliniMACS Prodigy Instruments, a set of time-tested processes enable different separation strategies to obtain NK cells from blood products. Depending on the process used, the output consists of highly enriched NK cells, pure or in combination with other therapeutically relevant cell types, like  $\gamma\delta$  T cells (fig. 1).

In clinical applications using allogeneic NK cells, the separation efficiency and product purity are of the utmost importance. Our two-step clinical-grade NK cell separation process efficiently removes T cells via  $CD3^+$  cell depletion and purifies NK cells via  $CD56^+$  cell enrichment. The resulting cell product consists of highly pure and viable NK cells, with a minimal presence of other immune cell types (fig. 3).



**Figure 3:** Efficient clinical-grade NK cell separation on the CliniMACS Prodigy Instrument. Output from processing a fresh non-mobilized leukapheresis product showed a mean log depletion of 4.3 for T cells and 2.6 for B cells. The mean recovery of NK cells was 43.4%, and the isolated NK cells were highly viable (94.4%) and pure. Most residual non-NK cells were monocytes.



Perform efficient, clinical-grade NK cell enrichment for downstream clinical applications.

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Discover other processes to enrich NK and other therapeutically relevant cells via targeted T cell depletion.

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# The tools for versatile NK cell manufacturing

## Efficient transduction and expansion

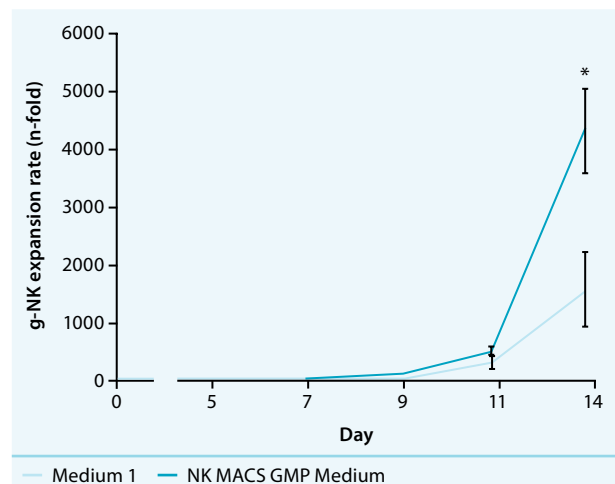
NK cells isolated on the CliniMACS Systems are ready for downstream processing. In the closed environment of the CliniMACS Prodigy Instrument, NK cells can undergo activation with a balanced cocktail of MACS® GMP Cytokines. Subsequent viral transduction can be enhanced using MACS GMP Vectofusin®-1. Both steps are carried out in an automated process that is flexibly programmable to fit your specifications. Internal data show that the transduced cells are highly viable and pure after expansion (Table 1).

Parameter	Seeded cells	Final cell product
Number of NK (CD3 <sup>+</sup> CD56 <sup>+</sup> ) cells	$1.08 \times 10^8 \pm 0.31 \times 10^8$	$1.71 \times 10^9 \pm 0.92 \times 10^9$
Number of transduced NK cells		$9.69 \times 10^8 \pm 4.40 \times 10^8$
NK cell purity (%)		99.01 ± 0.70
NK cell viability (%)		87.61 ± 4.34
Transduced cell frequency (%)		62.49 ± 17.34

**Table 1:** Synergies between instrument, consumables, and reagents lead to the reliable performance of NK cell transduction and expansion on the CliniMACS Prodigy Instrument. The final cell product is pure and viable, with a large proportion of transduced cells (>60%). The data shown are the average and standard deviation of seven donors. Transduction was performed using a BaEV LVV.

## Promoting NK cell growth

*Ex vivo* expansion of engineered or non-engineered NK cells is challenging. Miltenyi Biotec designed the NK MACS GMP Medium to specifically support NK cell growth and preserve their function (fig. 4). High-quality MACS GMP Cytokines – such as IL-2, IL-15, or IL-18 – synergize to create growth-promoting conditions for NK cell expansion within a GMP-grade setting.



**Figure 4:** A comparison of expansion rates of g-NK cells<sup>7</sup> using NK MACS GMP Medium or an alternative GMP-compliant medium. In the second week of cultivation, the expansion of g-NK cells is significantly enhanced by NK MACS GMP Medium. The values shown are the mean of seven donors with standard error bars (\* p < 0.05).

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Boost NK cell activation and expansion with fit-for-purpose culture reagents. Use MACS GMP Products.

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Learn about human g-NK cells and how they are efficiently expanded with Miltenyi Biotec reagents.

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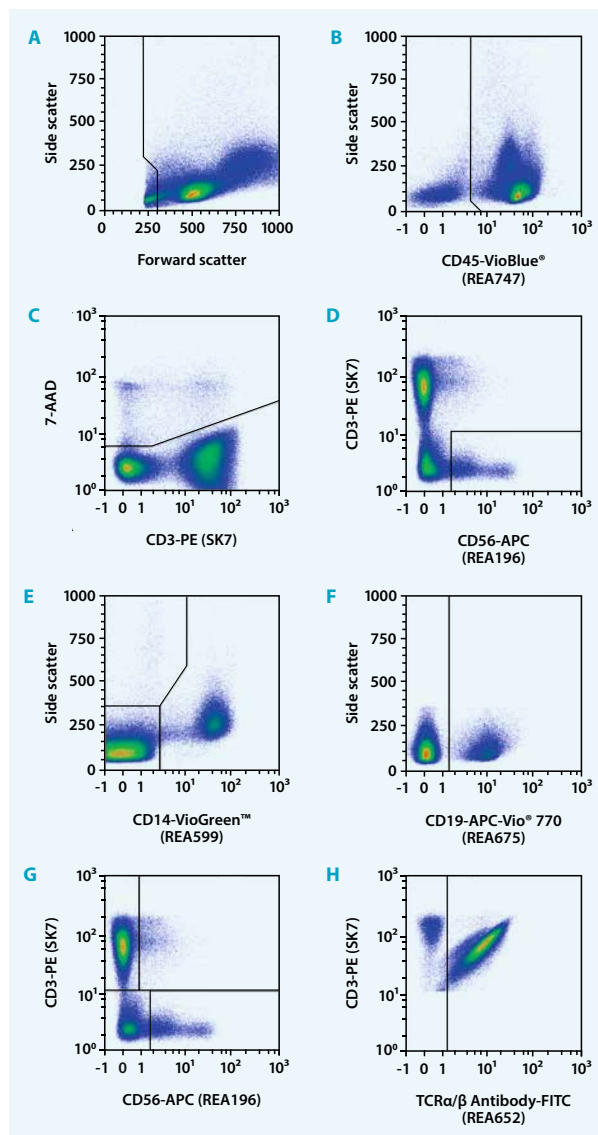
# Advance your product with certainty

## Seamless IPC/RT from the start

Incorporating reliable in-process control and release testing (IPC/RT) into NK cell manufacturing workflows streamlines quality, safety, and efficacy assessments of the final product.

Samples of a manufacturing run are easily collected from dedicated pouches in the CliniMACS Prodigy Tubing Set for quality control testing via flow cytometry. The specialized NK Cell Express Mode Package automates data acquisition and analysis on the MACSQuant® Analyzers. Using established multi-color panels of highly specific REAfinity® Recombinant Antibodies, this analytics platform ensures standardized and reliable results.

The NK Cell Express Mode Package is also used to determine the transduction efficiency of gene engineering steps, like in the manufacture of CAR NK cells (data not shown). Some contexts, like allogeneic adoptive cell therapy, may call for assessing the differential expression of killer-cell immunoglobulin-like receptors (KIRs) on donor NK cells. The NK Cell Express Mode package also facilitates simple KIR typing via flow cytometry for NK cell donor selection.



**Figure 5:** Flow cytometry analysis of a fresh leukapheresis sample from a healthy human donor prior to NK cell manufacturing. With automated gating and analysis, the NK Cell Express Mode Package determines the immune cell composition of the sample. (A) debris exclusion; (B) CD45+ cells; (C) viable cells; (D) NK cells; (E) monocytes and lymphocytes; (F) B cells; (G) T cells; (H) αβ T cells.



Automate IPC/RT analyses and simplify quality control with our NK Cell Express Mode Package.

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Discover further tools to standardize your analytical workflows and build reliable GCT analytics.

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# Everything you need in one hand

	CliniMACS Plus*	CliniMACS Prodigy*	MACSQuant Analyzer
Separation	CliniMACS CD3 and CD56 Product Lines		<b>In-process control</b> NK Cell Express Mode Package REAfinity Recombinant Antibodies CAR Detection Reagents**
Activation		NK MACS GMP Medium MACS GMP Cytokines	
Transduction		Viral vector (e.g., BaEV LVV) MACS GMP Vectofusin-1	
Expansion		NK MACS GMP Medium MACS GMP Cytokines	<b>Release testing</b> NK Cell Express Mode Package REAfinity Recombinant Antibodies CAR Detection Reagents** MACSPlex Cytokine Kits
Harvest		CliniMACS Formulation Solution	

\* Consult your Miltenyi Biotec representative to find the right tubing set for your process and instrument.

\*\* Enables analysis of CAR-engineered NK cells with trial-ready specificity and consistency.



See where and how NK cells are used.  
Read about therapy strategies using isolated and engineered NK cells.

► [miltenyibiotec.com/NKcell-engineering-refs](https://miltenyibiotec.com/NKcell-engineering-refs)

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In the EU, the CliniMACS System components are available as CE-marked medical devices for their respective intended use, unless otherwise stated. The CliniMACS Reagents and Biotin Conjugates are intended for *in vitro* use only and are not designated for therapeutic use or direct infusion into patients. The CliniMACS Reagents in combination with the CliniMACS System are intended to separate human cells. Miltenyi Biotec as the manufacturer of the CliniMACS System does not give any recommendations regarding the use of separated cells for therapeutic purposes and does not make any claims regarding a clinical benefit. For the manufacturing and use of target cells in humans, the national legislation and regulations – e.g. for the EU the Directive 2004/23/EC ("human tissues and cells"); or the Directive 2002/98/EC ("human blood and blood components") – must be followed. Thus, any clinical application of the target cells is exclusively within the responsibility of the user of a CliniMACS System.

In the US, the CliniMACS CD34 Reagent System, including the CliniMACS Plus Instrument, CliniMACS CD34 Reagent, CliniMACS Tubing Sets TS and LS, and the CliniMACS PBS/EDTA Buffer, is FDA approved as a Humanitarian Use Device (HUD), authorized by U.S. Federal law for use in the treatment of patients with acute myeloid leukemia (AML) in first complete remission. The effectiveness of the device for this indication has not been demonstrated. Other products of the CliniMACS Product Line are available for use only under an approved Investigational New Drug (IND) application, Investigational Device Exemption (IDE) or FDA approval.

MACS GMP Products are for *ex vivo* cell processing only, and are not intended for human *in vivo* applications. For regulatory status in the USA, please contact your local representative. MACS GMP Products are designed, manufactured and tested under an ISO 13485 quality management system and are in compliance with relevant GMP guidelines. They are designed following the recommendations of USP <1043> on ancillary materials. The manufacturing and testing of MACS GMP Products of biological origin are in compliance with EP chapter 5.2.12 for "Raw materials of biological origin for the production of cell-based and gene therapy medicinal products". CliniMACS, CliniMACS Prodigy, MACS, MACSQuant, the Miltenyi Biotec logo, REAfinity, Vio, VioBlue, and VioGreen are registered trademarks or trademarks of Miltenyi Biotec B.V. & Co. KG and/or its affiliates in various countries worldwide. Vectofusin is a registered trademark of Genethon. Copyright © 2025 Miltenyi Biotec and/or its affiliates. All rights reserved.