



Advanced Products for Cell Therapy

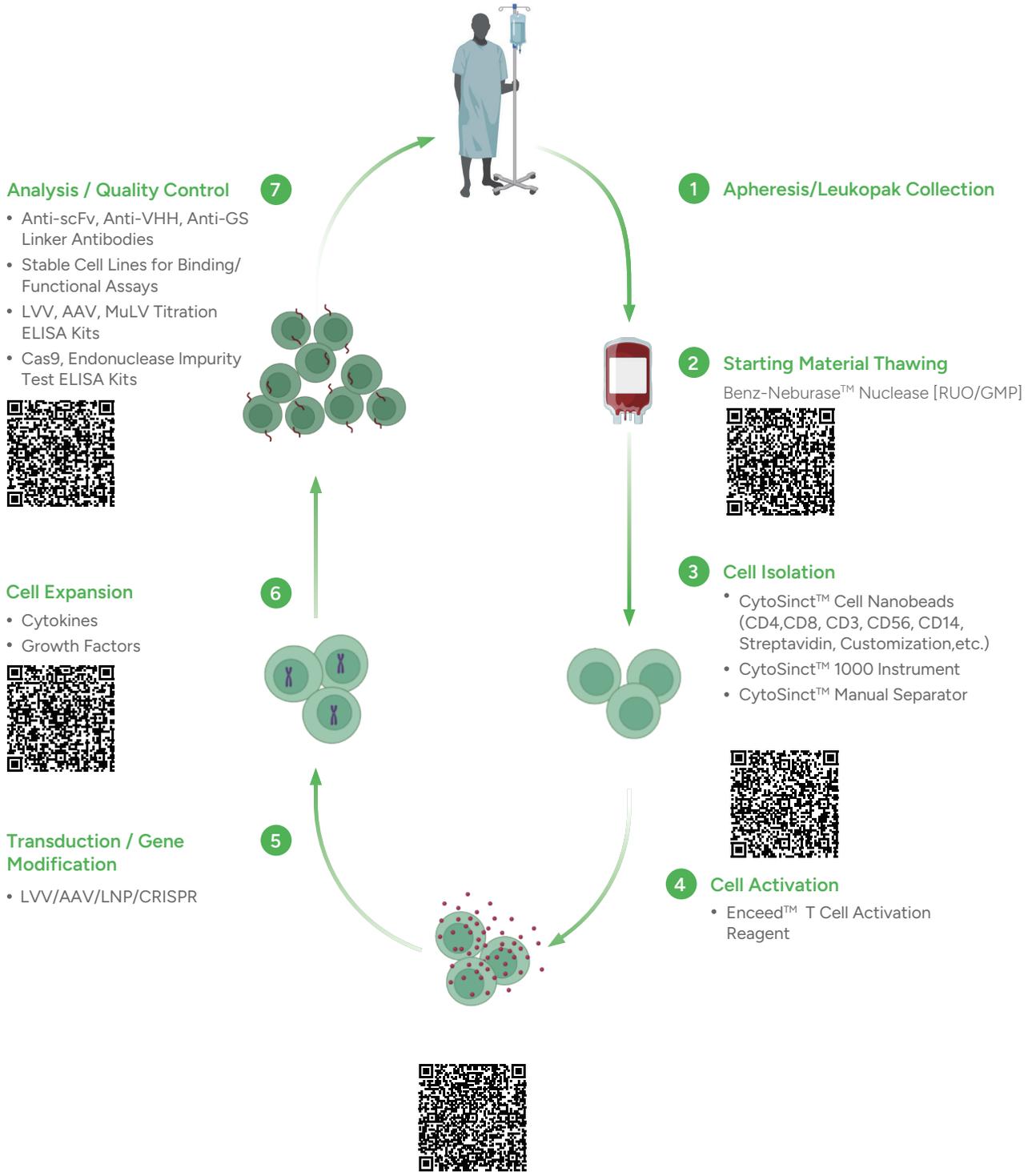
Application Note

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Cell Therapy Workflow Example and Ready-to-use Solutions

Seamless Scaling-up from RUO to GMP



Check out all Cell Therapy Ready-to-use Solution Here

OVERVIEW

Cell based therapies have emerged as one of the most promising approaches in modern medicine, offering novel solutions for a wide range of diseases, from cancer to autoimmune disorders and regenerative medicine. These therapies harness the power of the body's immune and regenerative systems, with various types of cells, including T cells, NK cells, macrophages, and stem cells, playing pivotal roles in targeted treatments. Advances in genetic engineering and in vitro expansion techniques have enhanced the efficacy of these therapies, with some already receiving regulatory approval and others showing promise in clinical trials. Below is an overview of key cell based therapies currently shaping the field, their mechanisms, and recent developments.

CAR T cell therapy has shown great promise in recent years. As of 2024, six CAR T cell therapies targeting hematological malignancies have been approved by the FDA since the first approval in 2017. Some of these therapies are now being used in earlier lines of treatment, expanding their target populations. Recently, CAR T cell therapy has also demonstrated encouraging clinical outcomes in autoimmune diseases^[1].

TCR T cell therapy represents another promising T cell based approach, particularly in the treatment of solid tumors. This is due to certain intrinsic advantages of TCRs, such as a target repertoire not limited to membrane proteins and their high antigen sensitivity^[2].

$\gamma\delta$ T cells, while accounting for less than 5% of the total T cell population, have emerged as a highly promising effector cell type for cancer immunotherapy. Their ability to recognize and kill transformed cells independently of HLA antigen presentation is key to their therapeutic potential. Current strategies for $\gamma\delta$ T cell therapies involve developing both autologous and allogeneic approaches, typically including in vitro expansion using cytokines and CD3 monoclonal antibodies, with potential incorporation of CAR engineering or gene modification to enhance chemotherapy resistance^[3].

Natural killer (NK) cells, a vital component of the innate immune system, play a key role in identifying and eliminating distressed cells, including tumor and virus infected cells. NK cells exert direct effector functions against these targets and support the broader immune response. Advances in receptor engineering have led to significant progress in NK cell based cancer immunotherapies^{[4],[5]}.

Monocyte derived, ex vivo generated macrophages have also gained attention in recent years, showing superior performance over stem cells in certain clinical trials. The development of chimeric antigen receptor (CAR) macrophages has further highlighted the potential of genetically engineered macrophages in advancing cell therapy^[6].

Tumor infiltrating lymphocyte (TIL) therapy is an adoptive cell transfer treatment, uses a patient's own immune cells to fight cancer. This involves harvesting TILs from the patient's tumor, expanding those with high specificity for tumor antigens in vitro, and reinfusing them to enhance antitumor responses. In 2024, TIL therapy received FDA approval for the treatment of relapsed or refractory melanoma and is currently being tested in clinical trials for other cancers^[7].

Stem cell therapy is a offers regenerative treatment options for various diseases and conditions. Hematopoietic stem cell transplantation (HSCT), commonly known as a bone marrow transplant, involves isolating stem cells from a healthy donor and infusing them into a patient with dysfunctional or depleted bone marrow to treat hematologic disorders or malignancies. Mesenchymal stem cell therapy has also gained worldwide attention due to the cells' multipotency in tissue regeneration and their strong immunosuppressive properties^[8]. This approach typically involves isolating and expanding cells in vitro before infusion back into the patient.

Despite the promising advancements in cell based therapies, several challenges remain. **Manufacturing scalability and high production costs are significant hurdles**, particularly in personalized therapies like CAR T and TIL, which require complex in vitro expansion processes. **Complex and time-consuming QC tests** also needs to be improved to enable timely batch-release.

GenScript is addressing these challenges by offering comprehensive solutions that streamline the manufacturing process, improve scalability, and reduce costs. Through innovations in **automated cell processing systems, gene editing technologies, and robust quality control protocols**, GenScript is helping to optimize cell therapy workflows and improve the accessibility and efficiency of these life life-saving treatments.

Cryopreservation and Thawing

Cell clumping is a major challenge in cell therapy manufacturing, impacting cryopreservation, thawing, and expansion by reducing viability and therapeutic efficacy. It complicates downstream processing, affecting cell isolation, quality, and scalability, while increasing contamination risks.

Benz-Neburase™ Nuclease For Safely Reducing Cell Clumping

Benz-Neburase™ *S. marcescens* Nuclease is a genetically engineered endonuclease derived from *Serratia marcescens*, designed for efficient digestion of all forms of DNA and RNA into 3-5 base nucleotides. It is highly effective in reducing nucleic acids without impacting cell viability, even during extended treatments. Available in both RUO and GMP grades, the tag-free Benz-Neburase™ offers a safe and efficient solution for reducing cell clumping, particularly when thawing frozen cell samples.

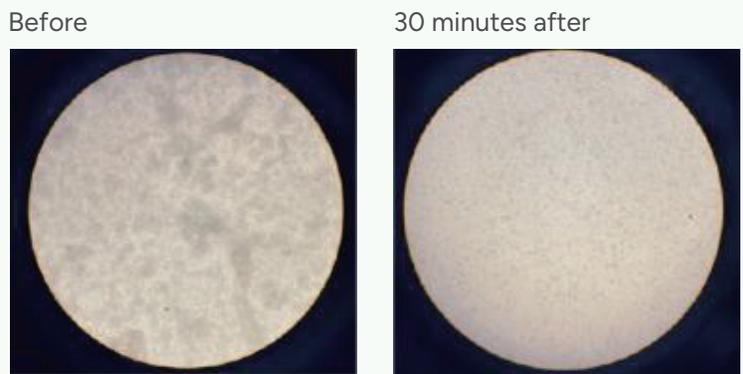


Figure 1: Benz-Neburase efficiently reduced cell clumping from SUP-T1 cell culture 30 minutes after treatment at 50 units/mL, 37°C.

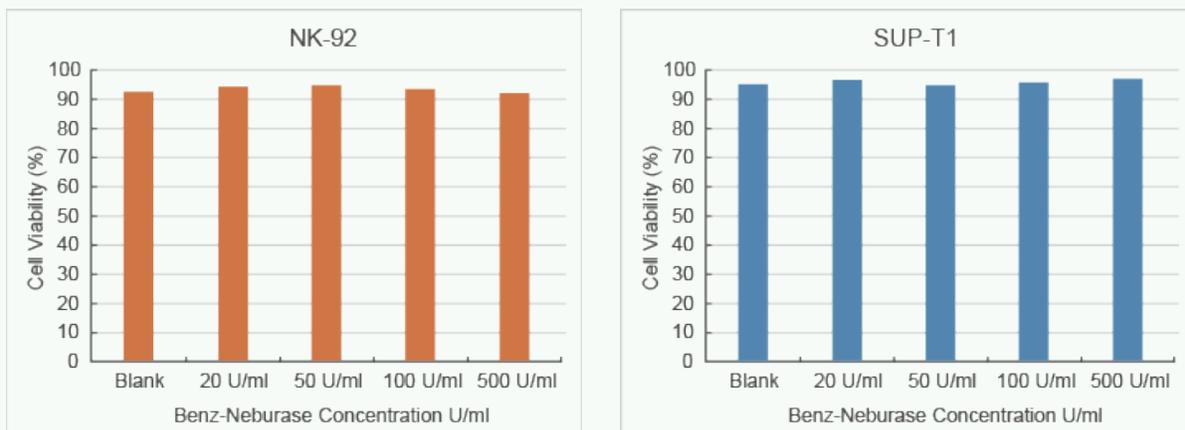


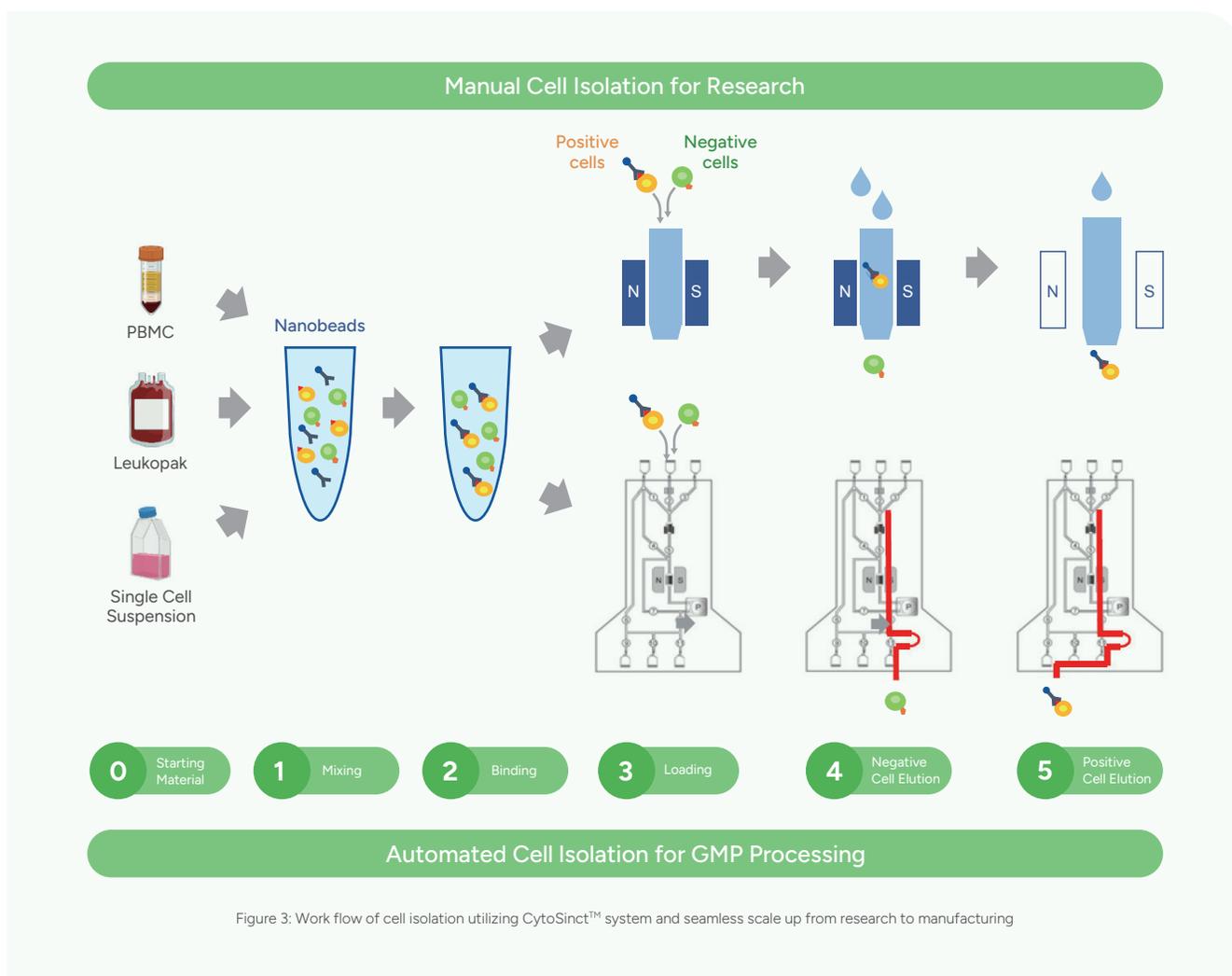
Figure 2: Benz-Neburase has minimal to no impact on the cell viability after incubation with each time of cell line over night at 20-500 units/mL, 37°C.

Cell Isolation

Cell isolation is a critical step in the development and manufacturing of cell-based therapies. Precise isolation of target cells based on surface markers ensures the purity and potency of the therapeutic product while minimizing contamination risks. Traditional methods, such as density gradient centrifugation and FACS, are labor-intensive, time-consuming, and may not scale well or achieve high specificity. Advanced immuno-magnetic technologies, using magnetic beads coated with antibodies, offer more efficient, scalable, and targeted cell isolation.

Cell Isolation with CytoSinct™ System

Leveraging GenScript's expertise in antibody development and magnetic bead production, CytoSinct™ Nanobeads are nano-sized, biodegradable, column-compatible, and do not require de-beading, ensuring efficient isolation of both common and rare cell populations. Available in RUO and GMP grades, CytoSinct™ Nanobeads, along with manual and automated instruments, support seamless scaling from research to manufacturing.



CD4+/CD8+ T Cell Isolation

Purified T cell subsets can enhance the biological activity of the final CAR T cell therapy product, in line with FDA recommendations^[9]. CD4+ and CD8+ combined isolation is one of the most commonly used strategies for T cell enrichment due to its ability to avoid T cell activation. The co-incubation and single-step elution of both CD4+ and CD8+ cells using CytoSinct™ Nanobeads significantly reduces processing time compared to sequential isolation (CD4+ followed by CD8+), while maintaining the same high level of purity. In addition, CD4+ cell isolation with CytoSinct™ Nanobeads has shown a trend of higher recovery rates compared to the current standard method.

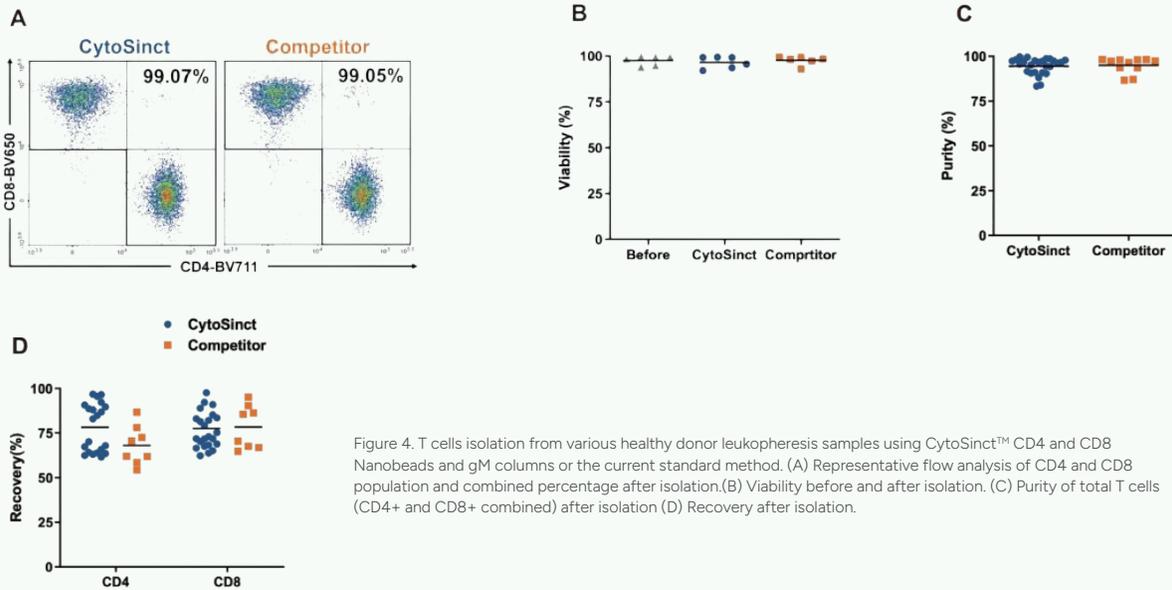


Figure 4. T cells isolation from various healthy donor leukopheresis samples using CytoSinct™ CD4 and CD8 Nanobeads and gM columns or the current standard method. (A) Representative flow analysis of CD4 and CD8 population and combined percentage after isolation. (B) Viability before and after isolation. (C) Purity of total T cells (CD4+ and CD8+ combined) after isolation. (D) Recovery after isolation.

NK Cell Isolation

Efficient enrichment of NK cells is critical for producing NK cell based therapies, such as CAR NK. However, isolating NK cells is more challenging than isolating T cells due to their relatively low abundance, typically around 10% in starting materials like leukopak or PBMCs. This process can be optimized by first depleting CD3+ cells, followed by enrichment of CD56+ cells, ensuring high purity without inducing NK cell activation.

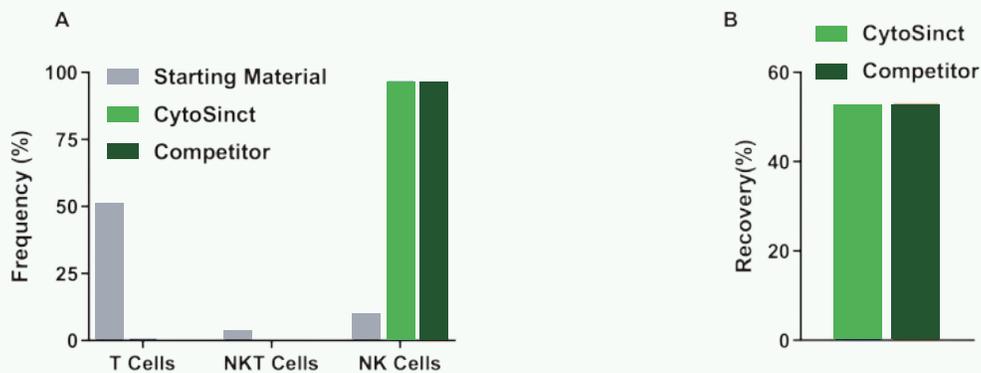


Figure 5. Representative flow analysis of NK (CD3-CD56+), NKT (CD3+ CD56+) and T cells (CD3+) following CD3+ cell removal and CD56+ cell enrichment from a leukopheresis sample using CytoSinct™ CD3 and CD56 Nanobeads and gM columns. (A) High purity of NK cells and sufficient removal of other cell types after isolation. (B) Recovery of NK cells (CD56+ CD3-) after isolation.

CD34+ Stem Cell Isolation

Isolating CD34+ stem cells presents a significant challenge due to their extremely low percentage, typically around 1%, in starting materials such as cord blood and mobilized peripheral blood. These sources also contain high levels of red blood cells and platelets, which can interfere with the isolation process. To achieve optimal results, PBMC enrichment is often required before magnetic isolation. Following this step, high purity stem cells can be efficiently isolated using CytoSinct™ CD34 Nanobeads.

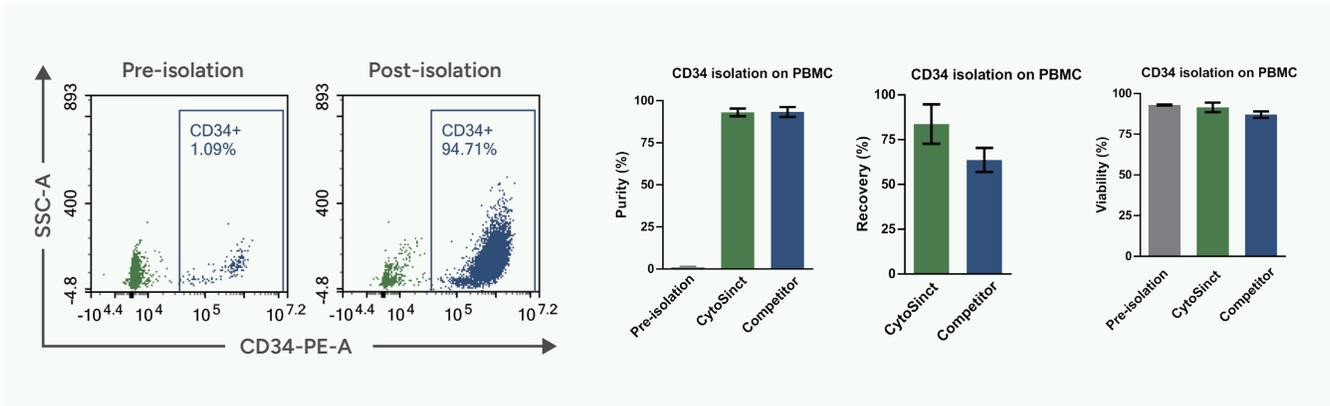


Figure 6. CD34+ cell line enrichment from a mix with PBCMs using CytoSinct™ CD34 Nanobeads and gL columns (A) Representative flow cytometry analysis before and after isolation (B) purity before and after isolation using CytoSinct™ CD34 Nanobeads or the current standard method.

One-Step TCR αβ Depletion For Allogeneic Cell Therapy

In allogeneic cell therapy, a key challenge is the risk of graft versus host disease (GvHD), where the transplanted cells attack the host, leading to adverse reactions. αβ T cell is the primary immune cell type that cause acute GvHD, and its removal at the end of the production process is an effective measure for allogeneic cell therapy to alleviate GvHD. Therefore, αβ T cell removal is a regulatory mandate by FDA for allogeneic CAR T cell therapy production. Additionally, αβ T cell depletion is being increasingly studied in allogeneic hematopoietic stem cell transplantation (allo HCT) to overcome the human leukocyte antigen (HLA) barrier in haploidentical (haplo) HCT, showing promising results^[10].

CytoSinct™ TCR αβ Nanobeads provide an efficient solution for removing αβ T cells from various allogeneic cell products, including but not limited to allogeneic CAR T, γδ T cells, stem cells and CAR NK samples, while preserving the remaining cell components. Unlike standard methods, CytoSinct™ TCR αβ Nanobeads come pre conjugated and ready to use, eliminating the need for additional steps such as labeling, washing, and resuspension. This simplified process saves 30 minutes to 2 hours of processing time, potentially reduces the use of one tubing set, and enhances cell recovery by 10- 20% (depending on whether using RUO or GMP process and the choice of cell washing instrument)

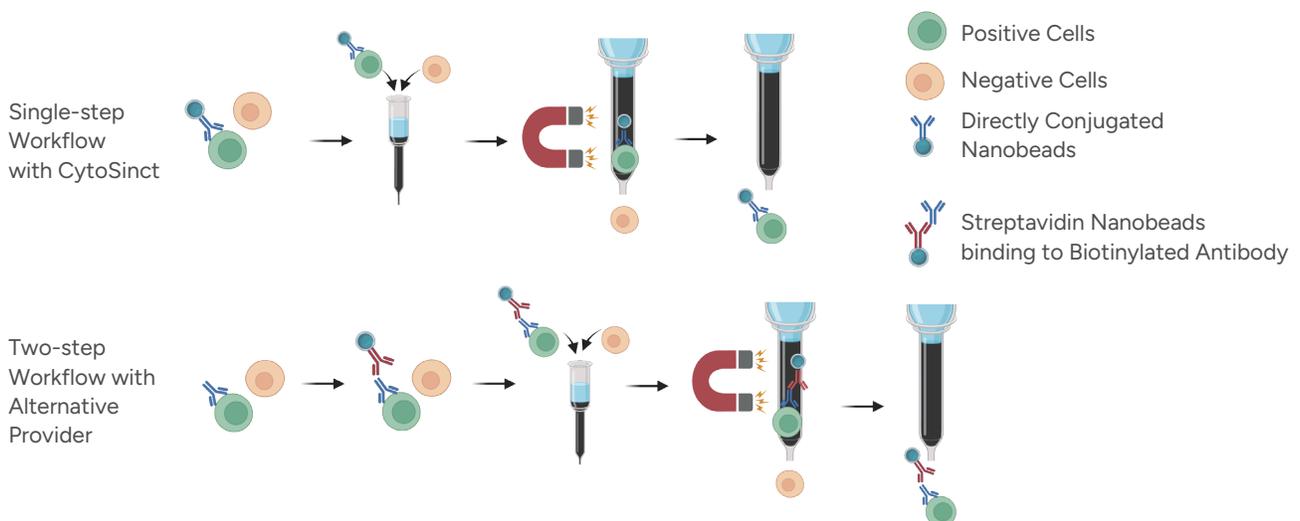


Figure 7. Component(s) and workflow of CytoSinct™ Tcr αβ beads or the current standard method

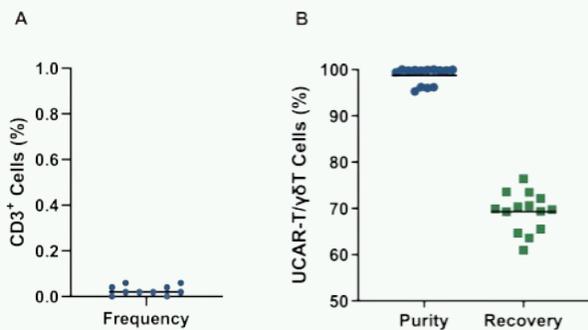


Figure 8. Flow analysis of various donor derived allogeneic CAR T cells or γ/δ T cells after TCR $\alpha\beta$ depletion using CytoSinct™ TCR $\alpha\beta$ Nanobeads and gL columns. (A) Percentage of CD3+ cells below 0.1% after TCR $\alpha\beta$ depletion. (B) Purity and recovery of CD3 cells after TCR $\alpha\beta$ depletion.

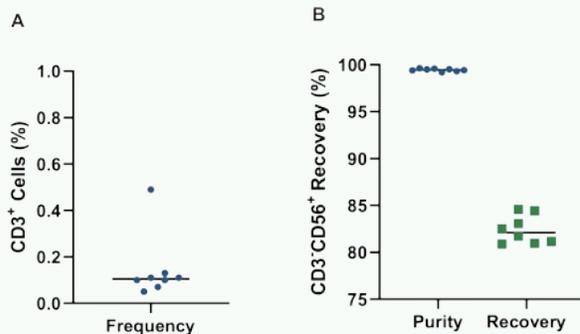
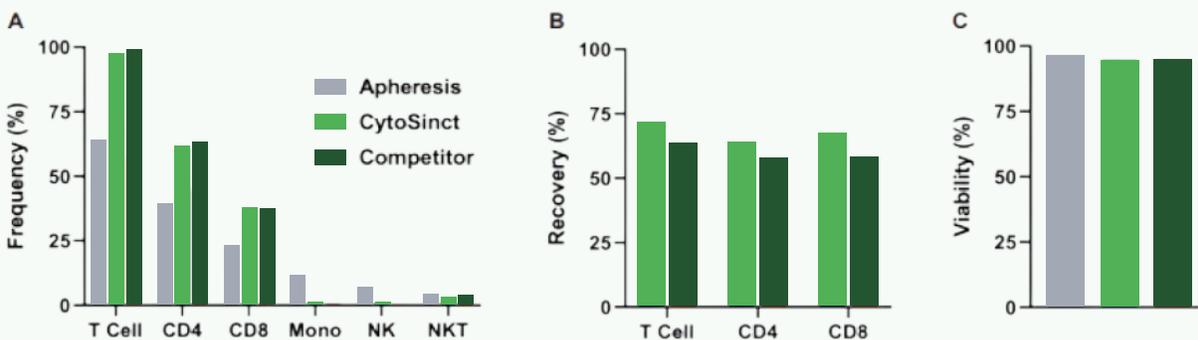


Figure 9. Flow analysis of various donor derived allogeneic CAR NK cells after TCR α/β depletion using CytoSinct™ TCR $\alpha\beta$ Nanobeads and gL columns. (A) Percentage of CD3+ cells mostly below 0.1% after TCR $\alpha\beta$ depletion. (B) Purity and recovery of CD3 cells after TCR $\alpha\beta$ depletion.

Closed, Automated Cell Isolation for Cell Therapy Manufacturing with Data Integrity

Automated, large scale cell isolation in a closed system is essential for successful GMP manufacturing and clinical application of cell therapy products. The CytoSinct™ 1000 is designed with 21 CFR Part 11 compliance, adhering to FDA regulations for electronic records and e-signatures, ensuring data integrity throughout the cell isolation process. Its customizable programming and alert functions streamline the operation, providing an efficient and reliable solution for automated cell isolation with peace of mind.



Test Groups	CytoSinct	Competitor
Instrument	CytoSinct™ 1000	Current Standard Method
Tubing Set	CytoSinct™ 1000 Tubing Set	
Reagent	CytoSinct™ CD4 and CD8 Nanobeads	CytoSinct™ CD4 and CD8 Nanobeads

Figure 10. T cells isolation from various donor leukapheresis samples using CytoSinct™ 1000 instrument and tubing set and similar instrument and tubing set from another provider, both with CytoSinct™ CD4 and CD8 Nanobeads. (A) Subpopulation analysis. (B) Recovery after isolation. (C) Viability before and after isolation.

Robotic Automation Option to Streamline Manufacturing

Cell therapy manufacturing faces the challenge of long process and risk of inconsistency. GenScript Biotech Corporation has partnered with Multiply Labs to fully automate the cell isolation phase of cell therapy manufacturing. By integrating GenScript's CytoSinct™ 1000 instrument with Multiply Labs' automation platform, this solution can fully automate, customizable solution that reduces the complexity and high costs of cell therapy manufacturing.

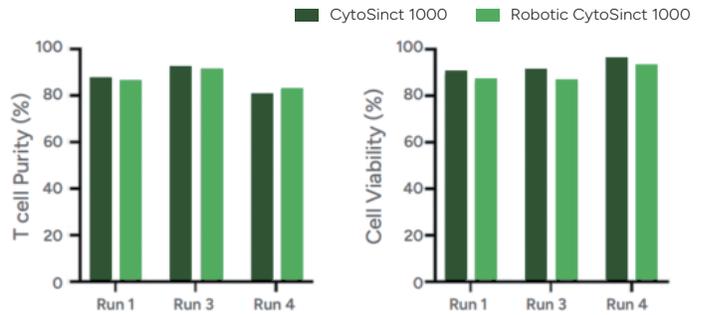


Figure 11. CytoSinct 1000 demonstrated comparable T-cell purity and viability across fully-integrated robotic and non-integrated semi-automated cell enrichment and separation processes.

T Cell Activation

Effective and gentle activation of T cells is critical for achieving high cell count in final product while also keeping young phenotype for persistence and clinical efficacy after infusion. Selected stem like or "young" T cells can significantly influence the biological activity of the final therapy, according to FDA guidelines^[9].

Optimized T Cell Activation with Enceed™ for Enhanced Memorial Phenotype & Consistency

Pre-conjugated with both anti-CD3 and anti-CD28 antibodies, Enceed™ T cell activation reagent is designed to activate T cells by engaging the T cell receptor (TCR) and the CD28 co-stimulatory module. Thanks to its nanometer size, unbound Enceed™ can be easily removed through cell washing or a medium change without the need for a magnet. T cells activated by Enceed™ present enhanced memory phenotype and greater expansion compared to those activated using standard methods. The consistent performance of Enceed™ across both RUO and GMP grades enables seamless scale up from research to manufacturing, ensuring its utility for large-scale T cell based therapies.

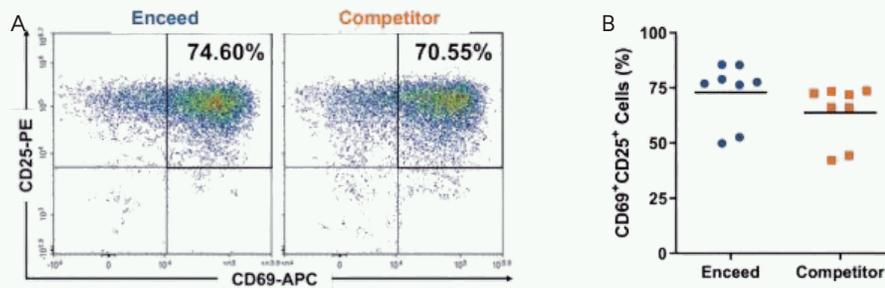


Figure 12. Flow analysis of cell activation markers CD25 and CD69 expression on T cells enriched from one (A) or multiple (B) healthy donor PBMC samples 2 days after treated with Enceed™ T cell activation reagent or using the current standard method.

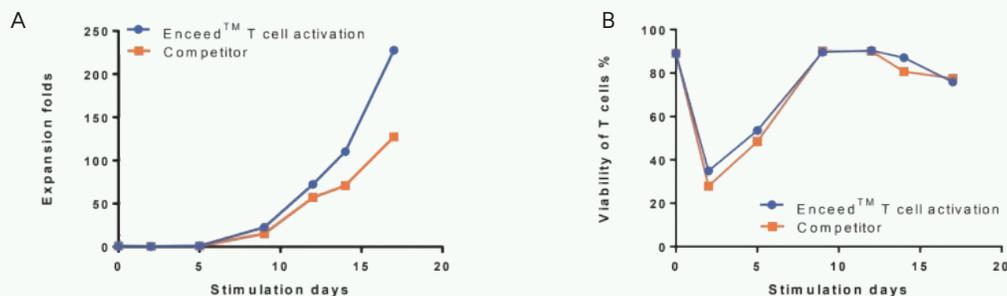


Figure 13. (A) Fold expansion and (B) viability of T cells enriched from a healthy donor PBMC after activated by Enceed™ or the current standard method and cultured with IL-2 for 17 days.

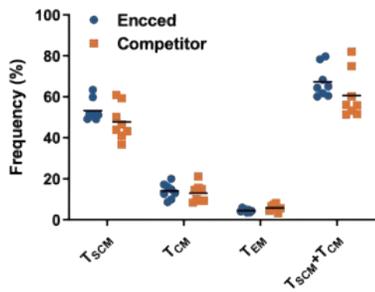


Figure 14. Enhanced memorial phenotype T cells from healthy donor PBMC samples 10 days after treated with Enceed™ and cultured with IL-2.

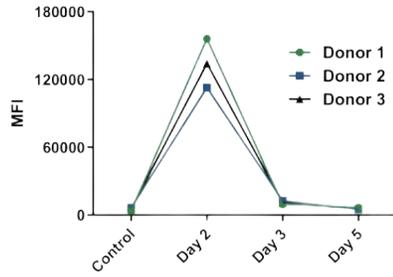


Figure 15. Flow cytometry analysis showing removal of cell surface Enceed™ by centrifugation 2 days after treatment.

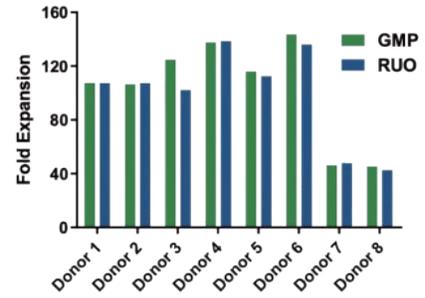


Figure 16. Consistent T cell expansion between GMP & RUO grade Enceed™ from various health donor derived PBMCs and after 10 days of culture with IL-2.

Cell Expansion

In cell therapy manufacturing, the in vitro expansion of therapeutic cells is a critical step that relies heavily on the use of cytokines and growth factors. These essential ancillary materials drive the growth and proliferation of various cell types. Cytokines like IL-2, IL-15, and IL-7 are indispensable for lymphocyte based therapies, such as CAR T, TCR T, NK cells, and tumor infiltrating lymphocytes (TILs)^[7]. Additionally, growth factors such as basic fibroblast growth factor (bFGF) have been shown to significantly enhance mesenchymal stem cell (MSC) expansion in vitro^[8].

Optimized Cell Expansion with GenScript Catalog Cytokines and Growth Factors

GenScript offers a comprehensive catalog of recombinant cytokines and growth factors at both RUO and GMP grades, designed to support efficient cell expansion for both research and large scale therapeutic manufacturing. These products are characterized by superior activity, excellent lot to lot consistency, and remarkably low endotoxin levels, ensuring reliable and effective cell growth in any application.

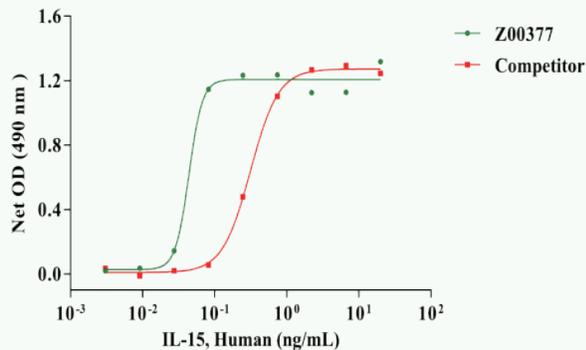


Figure 17. GenScript IL-15 (Cat.No. Z00377) ED50 < 0.5 ng/ml as determined by the dose-dependent stimulation of the proliferation of CTL-2 cells, corresponding to a specific activity of > 2.0 × 10⁶ IU/mg.

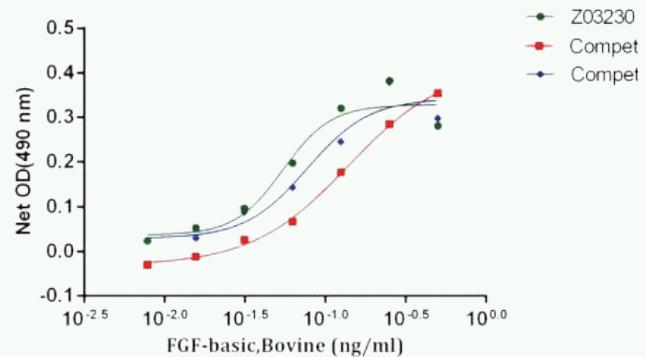


Figure 18. GenScript FGF-basic (Cat.No. Z03230) ED50 < 1.0 ng/ml as determined by the dose-dependent proliferation assay using 3T3 cells, corresponding to a specific activity of > 1.0 × 10⁶ units/mg.

Analytical/Quality Control in Cell Therapy

Flow Cytometry Analysis of CAR+ cells with MonoRab™ Anti-scFv and Anti-VHH Antibodies

Characterization of CAR expression, CAR+ cell count, and percentage is critical for CAR engineered cell therapy research, GMP manufacturing in process analysis, and lot release. The FDA recommends directly detecting CAR to accurately determine the percentage of CAR positive cells. Additionally, if surrogate proteins (co expressed genes) or broad specificity reagents (e.g., protein L) are used, their correlation with CAR expression should be evaluated[9]. Single chain variable fragments (scFv) and variable heavy chain domains (VHH) are commonly used CAR constructs in both research and clinical settings. MonoRab™ Anti-scFv and Anti-VHH antibodies are specifically developed for the detection of scFv and VHH CAR constructs, offering high specificity, affinity, and sensitivity across a variety of constructs. These antibodies provide non-blocking detection, ensuring that they do not interfere with the CAR's ability to bind its target cells, making them ideal for downstream application like functional assays.

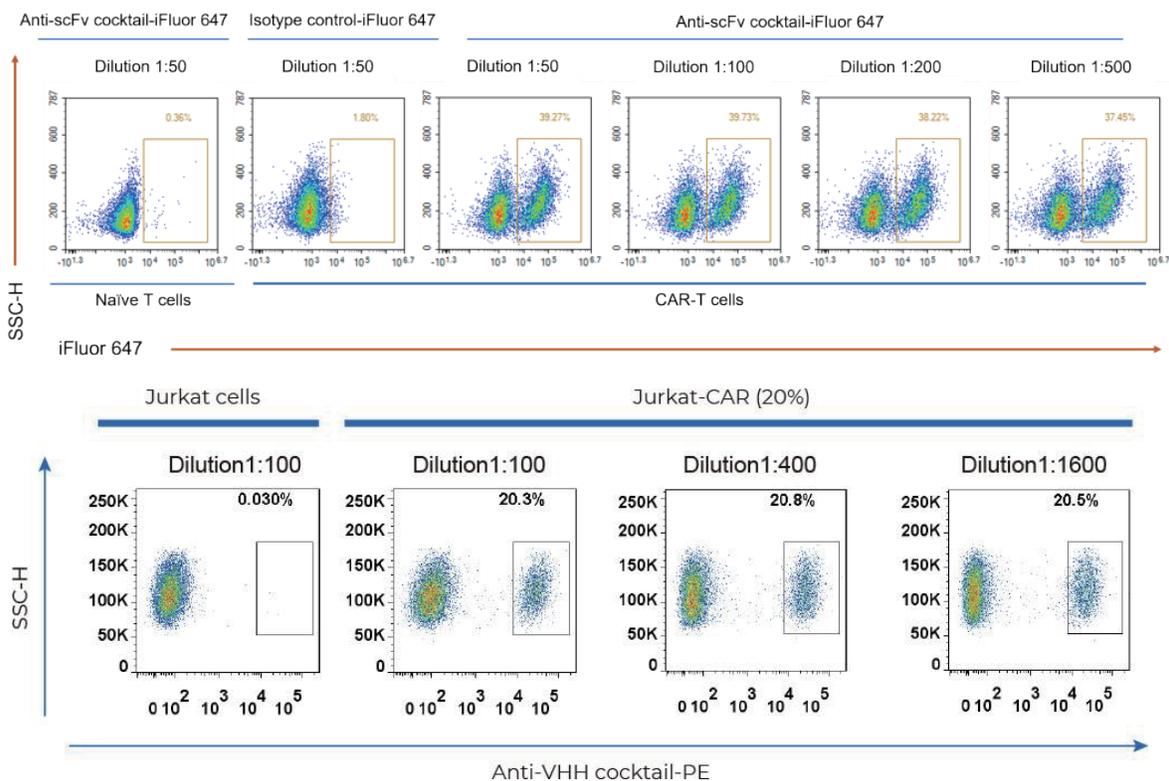
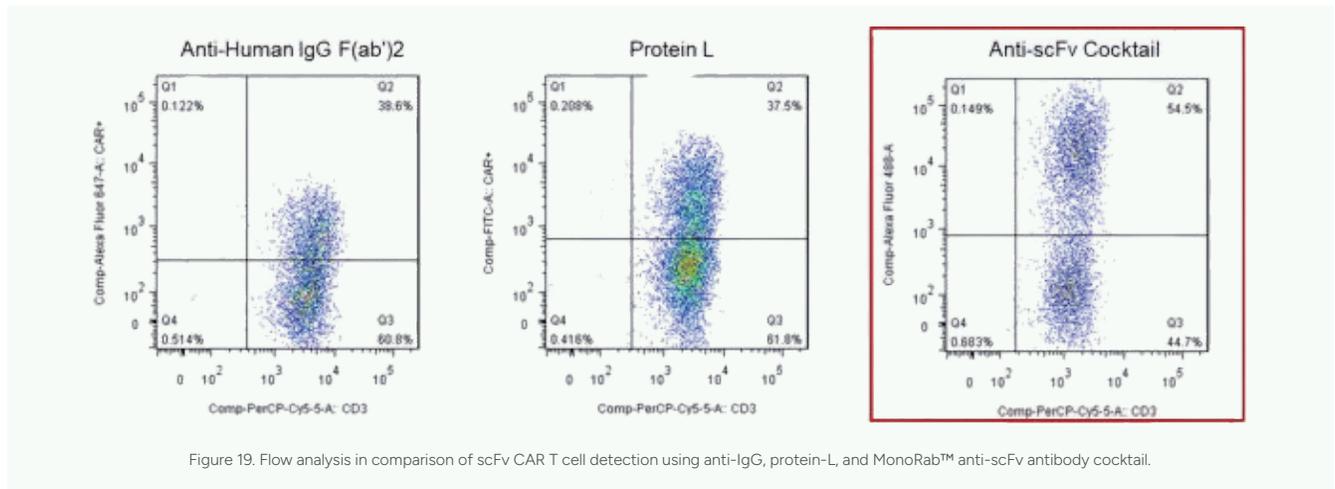


Figure 20. Flow analysis shows scFv CAR+ T cells and VHH CAR+ Jurkat cells detected by MonoRab™ Rabbit Anti-scFv Cocktail [iFluor 647] (Cat. No. A02288) and Anti-Camelid VHH Cocktail [PE] (Cat. No. A02018) with high accuracy at various dilution ratios.

Lentivirus, AAV and CRISPR are all common methods of transduction or genetic modification of the target cells. Depending on the method of choice, viral titration and impurity tests are critical QC considerations. GenScript provides a wide range of ELISA based QC kits with **< 2.5 hr protocols, ultra-high specificities and innovative technology** to streamline the QC workflow.



Accurate and Fast Lentiviral Titration with p24 ELISA Pro Kit

ELISA is a widely used method for the physical titration of lentiviral particles by quantifying p24 protein, offering a quicker and simpler alternative to measuring lentiviral RNA via qPCR. However, traditional ELISA may lack accuracy as it measures all p24 in the sample, including free p24 not incorporated into viral particles. The p24 ELISA Pro kit overcomes this limitation by using **proprietary magnetic bead technology to remove free p24, enhancing specificity and accuracy**. It supports a broad linear range of 30 to 2,000 pg /mL, reducing dilution requirements, and can be completed in just 2.5 hours, allowing for multiple runs per day as needed.

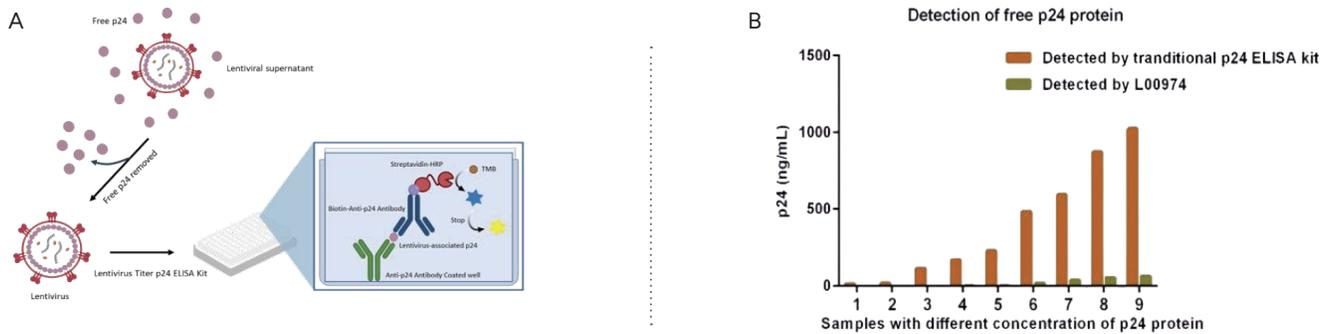


Figure 21. Highly accurate lentiviral titration by p24 ELISA assay through removal of free p24 protein from sample using magnetic beads. (A) schematic diagram of p24 Pro ELISA kit workflow. (B) Free p24 added to dilution buffer was detected by traditional P24 ELISA kit but not p24 Pro kit.

Streamlined Multi-serotype AAV Titration with AAVX ELISA Kit

Adeno-associated virus (AAV) is a leading gene delivery vector driving the development of innovative cell and gene therapies. Analytical development and QC for AAV can be complicated due to the different AAV serotypes. Leveraging strong antibody development platform, GenScript provides AAVX ELISA Titration Kits that can detect various AAV serotypes with high sensitivity and reproducibility within one kit, significantly reducing the complexity while maintaining high accuracy and fast workflow, empowering cell and gene therapy developers to streamline vector characterization and accelerate therapeutic development.

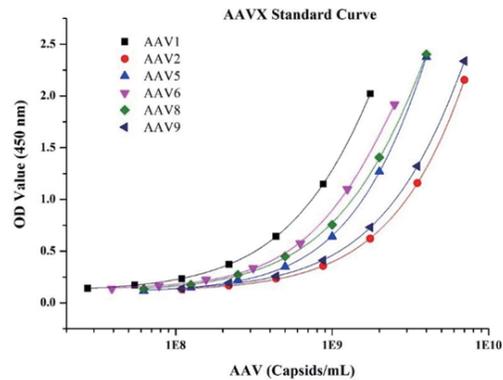


Figure 22. Representative standard curve showing wide detection range for various AAV serotypes with AAVX ELISA kit (Cat. No. L01035)

Broad Recognition with Cas9 Impurity ELISA Test

CRISPR-Cas9 is driving next-generation cell therapies, but residual Cas9 protein poses risks to safety and product quality. Cas9 comes with different orthologs and engineered version for specific gene editing needs. Accurate detection of the different variants is therefore essential in development and manufacturing.

GenScript's **ultra-sensitive Cas9 Residual Detection ELISA Kit** offers broad recognition of Cas9 variants and NLS (nuclear localization signals), enabling reliable safety assessment and process monitoring to advance gene and cell therapy development.

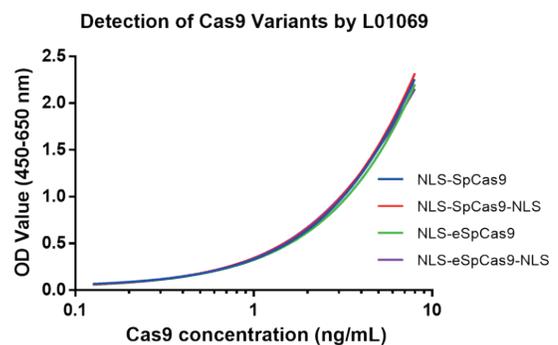


Figure 23. Different Cas9 variants and NLS types/numbers/positions can be detected by Cas9 ELISA Kit (Cat.No. L01069).

Stable Cell Lines for QC

Single clone-derived stable cell lines provide a consistent and well-characterized reference standard for cell therapy QC, ensuring reproducibility across assays and manufacturing batches. By offering genetic stability and uniform expression profiles, they enable reliable assessment of product potency, safety, and comparability throughout development and production.

Leveraging strong decades of expertise in gene editing and cell biology, GenScript offers 100+ over-expression stable cell lines for binding assays and reporter cell lines for functional assays. All the cell lines are single-clone derived and tested to ensure 16+ passages of stability, with tracibility documents ready to support your next regulatory application.

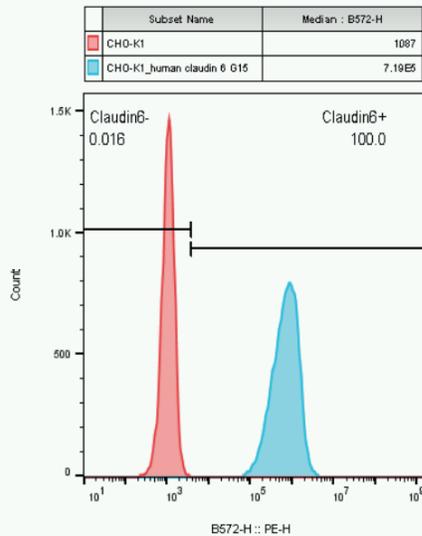


Figure 24. Flow cytometric analysis of CHO-K1/human Claudin 6 cells stained with anti-human Claudin 6 antibody (R&D, Cat. No.: MAB3656) after cell digestion using Accutase Cell Dissociation Reagent.

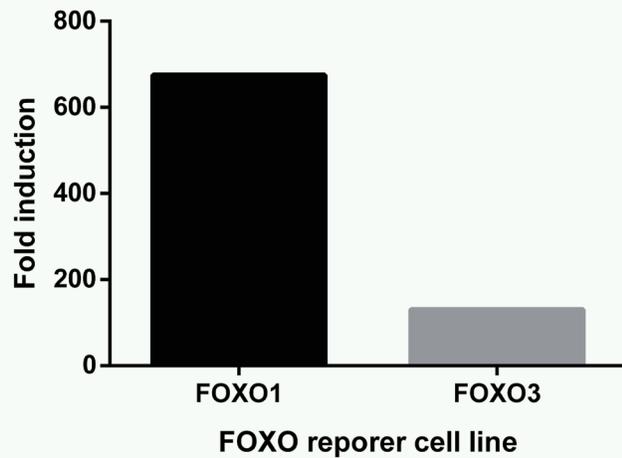


Figure 25. FOXO Reporter cells were transiently transfected with FOXO1 or FOXO3 plasmids using liposomes and incubated for 48 hours. After incubation, the cells were plated and incubated at 37°C, 5% CO₂ for 18 hours, luciferase substrate reagent was added and luminescence measured by PHERAstar. Data was analyzed by GraphPad Prism® software.

Regulatory Support



GenScript Biologics Manufacturing Center for GMP Grade Products

The GenScript Manufacturing Center in Zhenjiang China, spans over 100,000 square meters. The center is designed and constructed to fully comply with the good manufacturing practice (GMP) standards set by the FDA, EMA, PMDA, and NMPA. This state of the art facility is equipped to meet the demands of Phase I to III clinical sample production, as well as commercial scale manufacturing.

GenScript's quality management system is certified to ISO 13485 standard, ensuring rigorous processes for the design, development, manufacture, and distribution of GMP products. Drug Master Files (DMF) for GenScript's GMP grade products have been submitted to the FDA, supporting Investigational New Drug (IND) applications in the U.S.

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