Application Note

Robust T Cell Activation and Efficacy for Cell Therapy with Enceed™ T Cell Activation Reagent

Introduction

T cell activation is a pivotal step in the manufacturing of cell therapies, particularly in the development of T cell therapies, like chimeric antigen receptor (CAR) T cell therapies and TCR T cell therapies. Effective activation of T cells not only initiates their proliferation and functional programming but also directly influences transduction efficiency, phenotype, and therapeutic efficacy. T cell activation reagents can be designed to mimic natural antigen-presenting cell signals via CD3 and co-stimulatory CD28 engagement. The quality and consistency of these reagents play a crucial role in determining the success of downstream processes such as gene modification, expansion, and memory phenotype retention.

Importantly, activation reagents that do not require magnetic removal streamline the manufacturing workflow by reducing processing steps, minimizing cell loss, and preserving cell viability. In addition, eliminating the removal step reduces the risk of process-related impurities and avoids the need for additional quality control (QC) testing to confirm bead clearance - enhancing both operational efficiency and regulatory compliance in GMP manufacturing environments.

To address all of these critical needs, GenScript developed **Enceed™ T cell Activation, human (GMP)**, designed to deliver high activation efficiency, streamlined processing, with complete

regulatory compliance to advance clinical development and commercialization of T cell therapies.

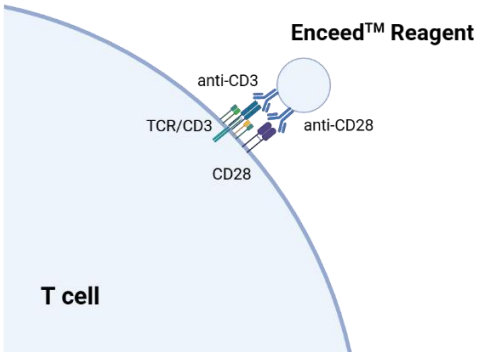

Figure 1. Schematic diagram of Enceed™ T cell Activation Reagent

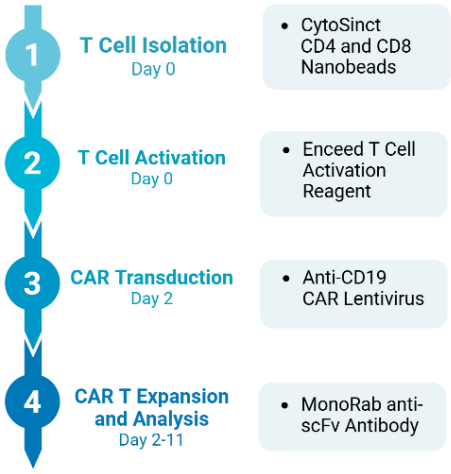

Figure 2. Workflow of CAR-T cell production and analysis

Materials and Methods

CD4+/CD8+ T cell Isolation

Frozen PBMC from 2 healthy donors were

removed from liquid nitrogen, thawed in 37°C water bath, centrifuged in T cell culture media, at 300 × g RCF for 5 min, and resuspended in PBS/EDTA Buffer containing 0.5% HSA, T cell were then isolated with CytoSinct™ CD4 Nanobeads and CD8 Nanobeads (GenScript). T cell purity, recovery and viability were analyzed by Agilent NovoCyte Flow Cytometers and Cellometer™ K2 fluorescent cell counter.

T cell Activation

Enriched T cells were plated at 1 × 10^6 T cell/mL in complete medium with IL-2 at 300 IU/mL, and then treated with Enceed™ T cell Activation, human (GMP) (GenScript), at 40 µL/1 × 10^6 enriched $CD4^+$/$CD8^+$ T cells respectively and stimulated for 48 h in a 37 °C, 5% CO_2 incubator.

T cell Transduction

Enriched $CD4^+$/$CD8^+$ T cells activated with Enceed™ T cell Activation, human (GMP), were transduced on Day 2 with a lentiviral vector encoding anti-CD19 CAR at MOI of 2. The transduction was done by centrifugation of the cell and virus mix at 1000 × g for 30min, and then incubated in a 37°C incubator with 5% CO_2. After 24h, 2 times the volume of fresh complete medium was added to the cell culture to quench the transduction.

CAR+% Detection

Transduction efficiency was analyzed by CAR expression on cell surface. 5 × 10^5 transduced T cells were resuspended in 100 µL of PBS. Anti-

scfv-PE (GenScript) was added and incubated at 4 °C for 15 min, washed with PBS twice, resuspended in PBS and with 7AAD staining solution at a ratio of 100:1, and then detected by Agilent NovoCyte Flow Cytometers.

CAR-T cell Expansion

Transduced T cells were plated at 5 × 10^5 cells/mL in complete media with 300 IU/mL IL-2 for expansion after transduction. T cell culture was monitored at different time points to determine cell density and viability. Fresh complete medium was added every 2-3 days to maintain a live cell density of 0.5-1 × 10^6/mL.

Killing Efficacy

Daudi-luc cells were plated in 96-well plates at 4000 cells/50 µL and CAR-T cells were added at various E:T ratios from 40:1 to 5:1 with the total volume of each well at 100 µL, and then cultured in a CO_2 incubator at 37 °C for 16 - 24h, and then treated with 50 µL ONE-Glo™ (Promega) for 5 min to avoid the light reaction. RLU value was then detected in the enzyme marker using Varioskan LUX microplate reader (ThermoFisher), and the killing efficiency was calculated according to the RLU value.

Memory Phenotype

Selected stem like or “young” T cells can significantly influence the biological activity of the final therapy, according to FDA guidelines [1].

5 × 10^5 transduced T cells were resuspended in 100 µL of PBS, and treated with Anti-CD3-BV785, Anti-CD45RA-APC, Anti-CD45RO-FITC, Anti-CD27-PE/CY7, Anti-CD197-PB, Anti-CD62L-APC/Cy7, Anti-CD95-PE at 4°C for 15 min, washed with PBS twice, resuspended in PBS with 7AAD staining solution at a ratio of 100:1, and then analyzed by Agilent NovoCyte Flow Cytometers.

Results and Discussion

T cell Isolation

T cells were isolated from healthy donor PBMCs using CytoSinct™ CD4 Nanobeads, human, and CytoSinct™ CD8 Nanobeads, human.

High purity (95.25%), recovery (81.3%) and viability (97.7%) were achieved after isolation.

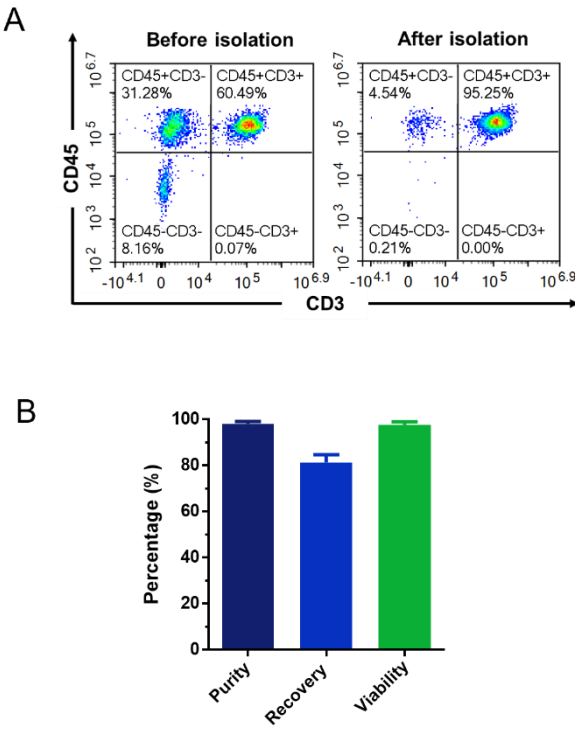

Figure 3. T cell isolation from healthy donor PBMC samples using CytoSinct™ Nanobeads (GenScript). (A) Representative flow analysis. (B) Average of purity, recovery and viability post-isolation from two different healthy donor PBMC samples.

Transduction Efficiency

Purified CD4⁺/CD8⁺ T cells were stimulated by Enceed™ T cell activation, human (GMP) and

competitor's activation reagent for 48h followed by anti-CD19 CAR lentivirus transduction, and anti-

CD19 CAR⁺ cell percentage was tested after 3, 6 and 11 days by FACS, respectively. Higher anti-CD19 CAR⁺ cell percentage was observed in T cells activated by Enceed™ than the competitor at both 3, 6 and 11 days after lentivirus transduction of T cells.

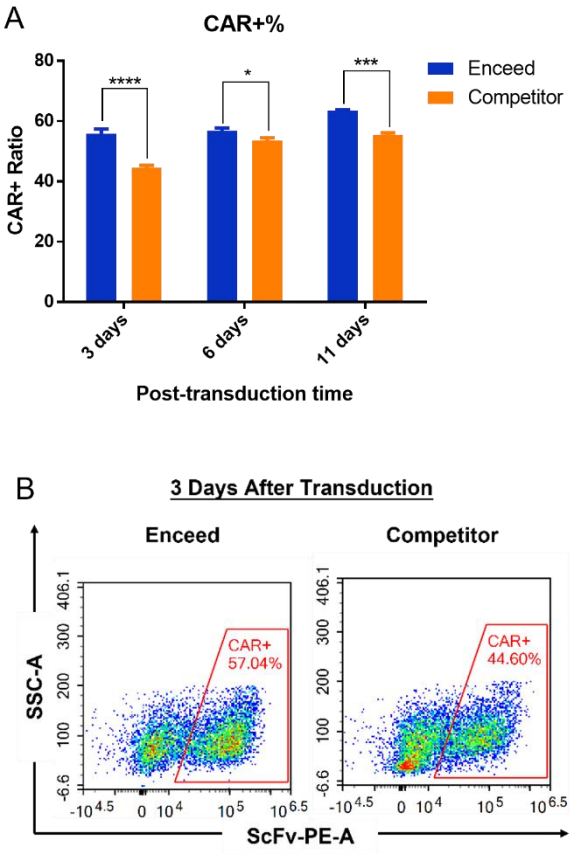

Figure 4. Transduction efficiency at various timepoints (A) and representative flow analysis (B) in CAR-T cells activated with Enceed™ or another provider's product.

Robust Expansion and High Viability

Purified CD4⁺/CD8⁺ T cells were stimulated by Enceed™ T cell Activation, human (GMP) and competitor's activation reagent for 48 h and then transduced with anti-CD19 CAR lentivirus.

Significantly higher fold-expansion was observed

starting from Day 11 compared to competitor. Viability was same on most timepoints tested and

higher on Day 12.

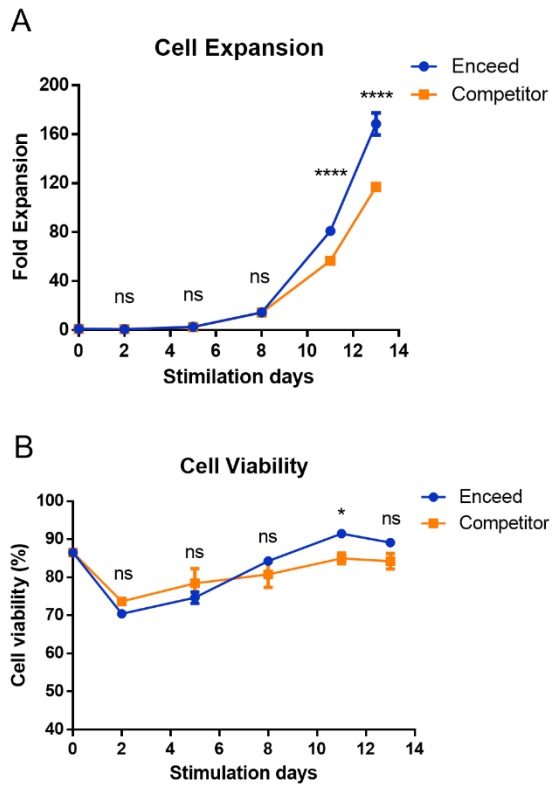

Figure 5. Cell expansion and viability post-activation and throughout expansion in comparison with another provider's product.

Memory Phenotype

Activated T cells were transduced with anti-CD19 CAR lentivirus 48h later, and T cell memory phenotype was analyzed 11 days after. Percentage of T_{SCM} and T_{SCM}+T_{CM} in CAR-T cells activated by Enceed™ were significantly higher than the competitor.

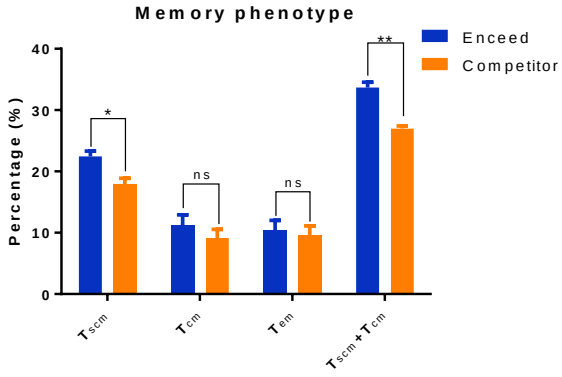

Figure 6. Flow analysis of memory phenotype in CAR-T cells 11 days after activation in comparison with another provider's product.

Killing Efficacy

11 days after transduction, Daudi-luc cells were cultured with CAR-T cells activated by Enceed™ or the competitor's reagent at different E:T ratios for 18 h. And then ONE-Glo™ was added, and the luminescence levels were detected in an microplate reader (Varioskan LUX, ThermoFisher). Killing efficacy was calculated based on the luminescence reduction compared to the Daudi-luc cells cultured without CAR-T. T cells stimulated by Enceed™ presented significantly higher efficacy against the target cell Daudi-luc at E/T of 20:1, 10:1 and 5:1 compared to the competitor.

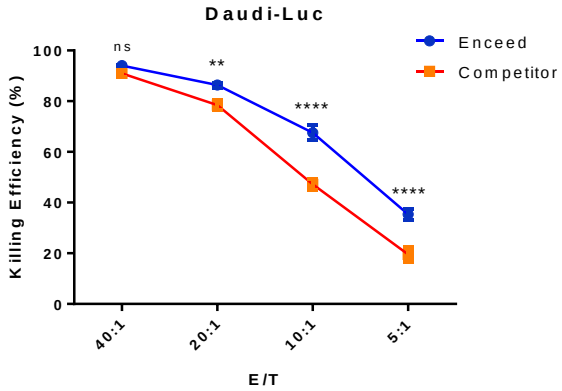

Figure 6. Killing efficacy of CAR-T cells against Daudi-Luc cells.

Conclusion

Enceed™ T cell Activation, human (GMP), demonstrated superior performance in generating functional CAR-T cells compared to the competitor. $CD4^+$/$CD8^+$ T cells activated with Enceed™ showed significantly enhanced activation and expansion, enabling efficient lentiviral transduction and robust CAR expression. Functional assays revealed higher cytotoxicity against Daudi-luc target cells, indicating improved killing efficacy. Moreover, memory phenotype analysis revealed a higher proportion of T cells expressing markers associated with central memory ($CD45RA^-$, $CD27^+$, $CD62L^+$) and stem cell memory ($CD45RA^+$, $CD45RO^-$, $CD27^+$, $CD62L^+$, $CD197^+$), consistently with higher fold-expansion and higher efficacy, suggesting that Enceed™ promotes the development of a durable and long-lived CAR-T cell population. These results support Enceed™ as a powerful and consistent solution for advanced T cell engineering and adoptive cell therapy applications.

References

[1] Considerations for the Development of Chimeric Antigen Receptor (CAR) T Cell Products Guidance for Industry. FDA, 2024 Jan.

Ordering Information

Product	Package Size	Capacity	Cat. No.
Enceed™ T cell Activation, human (GMP)	4 mL	Up to 2×10^8 T cells	L00935
CytoSinct™ CD4 Nanobeads, human (GMP)	7.5 mL	Up to 4×10^{10} total cells	L00932-7.5
CytoSinct™ CD8 Nanobeads, human (GMP)	7.5 mL	Up to 4×10^{10} total cells	L00933-7.5
CytoSinct™ CD3 Nanobeads, human (GMP)	7.5 mL	Up to 4×10^{10} total cells	L00934
CytoSinct™ CD34 Nanobeads, human (GMP)	7.5 mL	Up to 6×10^{10} total cells	L00948
CytoSinct™ CD56 Nanobeads, human (GMP)	7.5 mL	Up to 4×10^{10} total cells	L00949
CytoSinct™ TCR αβ Nanobeads, human (GMP)	7.5 mL	Up to 2×10^{10} total cells	L00952
CytoSinct 1000	1 unit	Compatible with CytoSinct™ 1000 tubing set products	D00023
CytoSinct TS Tubing Set (GMP)	1 set	Up to 6×10^{10} total cells	D00029
CytoSinct LS Tubing Set (GMP)	1 set	Up to 1.2×10^{11} total cells	D00030
CytoSinct DTS Tubing Set (GMP)	1 set	Up to 1.2×10^{11} total cells	D00031
CytoSinct™ PBS/EDTA Buffer (GMP)	3 x 1L	NA	B00064