VELSSON
AN INNOVEL COMPANY

IMMUNOVESSEL Cell Culture

ImmunoVessel, flourish your cell !

INNOVEL Cell Culture ImmunoVessel

ImmunoVessel™ suspension flasks are designed to provide consistent cell growth and reproducible results every time for any size application in production and research & development.

ImmunoVessel™ series ideal for any cell growth and amplification process and provides superior results with wide rande of human and animal cell lines, including: T Cell、CAR-T、TCR-T、TIL、NK、CAR-NK、CIK、K562、Jurkat、Raji & more.

Recommended seeding density ~5E+05 /mL, minimum seeding density 2E+05 /mL.

Cultivation Volume
10~25mL
Est. Cell Yield
2.5~4E+08

1

▲
ImmunoVessel-30

ImmunoVessel-220
▼

Cultivation Volume
50~180mL
Est. Cell Yield
1.8~3E+09

2

Cultivation Volume
50~360mL
Est. Cell Yield
3.6~5.5E+09

3

▲
ImmunoVessel-440

ImmunoVessel-500
▼

Cultivation Volume
50~400mL
Est. Cell Yield
4~6E+09

4

Cultivation Volume
150~1000mL
Est. Cell Yield
1~1.5E+10

5

▲
ImmunoVessel-1000

- Manufactured with medical grade materials for superior transparency and biocompatibility.
- Patented double layered permeable membrane technology optimizes gas exchange for superior cell growth with cultivation density quotient over 1E+07 /mL.
- Uniquely positioned top filter is ergonomically placed to prevent accidental membrane wetting and lowers the risk of contamination.
- Supernatant removal can be performed without centrifugation to avoid damage to cells and allows optimal use of media (ImmunoVessel™ – 500 & 1,000).
- Space saving design ideal for static cultures and maximizes incubator space.
- Non-pyrogenic & sterilized with gamma irradiation.

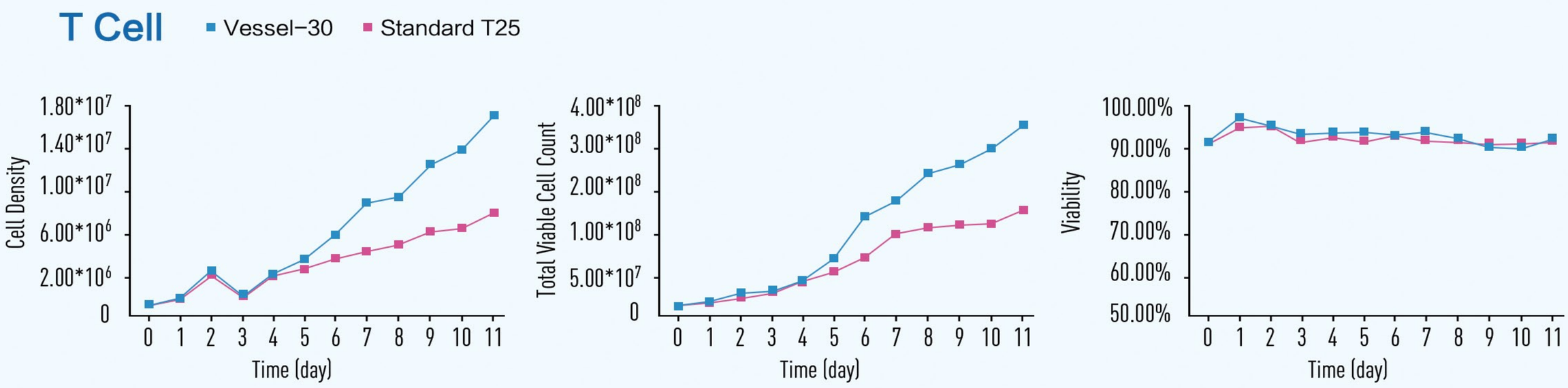
ImmunoVessel-30 Culture Mini-Bottle

The unique cap design with a double-layered inner sleeve membrane and a breathable outer layer promotes superior cell growth and health.

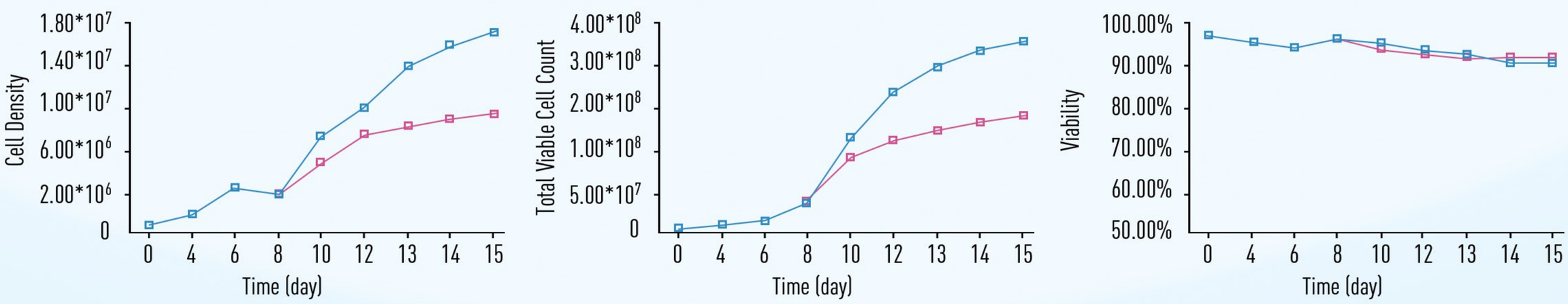
01 Culture amplification of T/CAR-T Cells in ImmunoVessel-30 vs. Standard T25

The recommended culture volume is 10-25mL and the recommend seeding density is ~5E+05/mL(minimum seeding density, 2E+05/mL).

T Cell



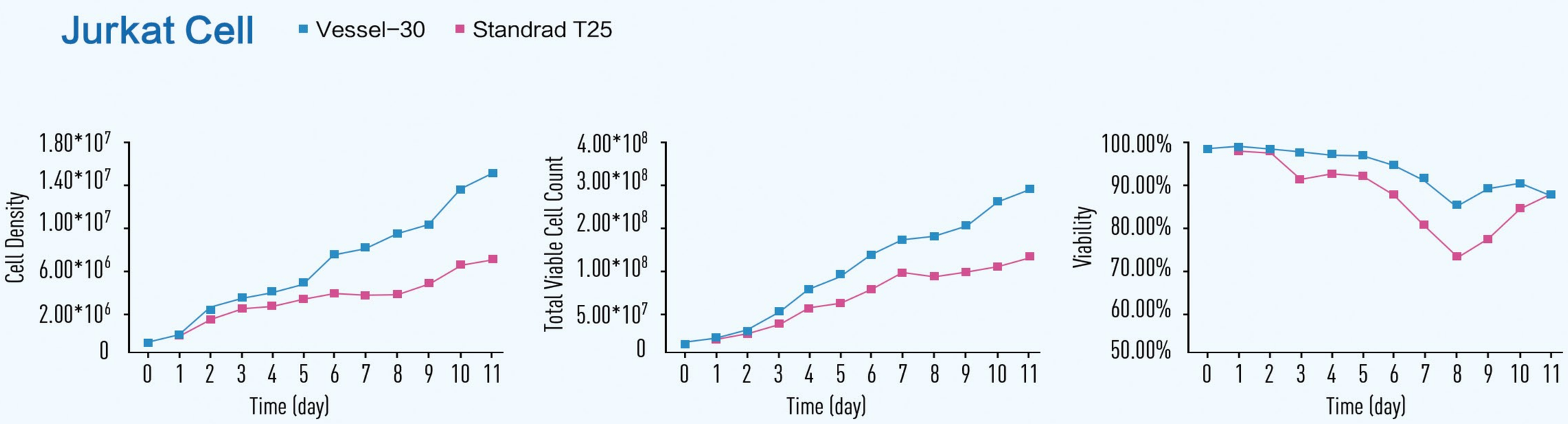
CAR-T Cell



ImmunoVessel-30 flasks attained high level viable cell density over 1.6E+07/mL with T/CAR-T cells achieving 2X higher yield than standard T25 flasks.

02 The Jurkat cells cultured and expanded in ImmunoVessel−30 and standard T25

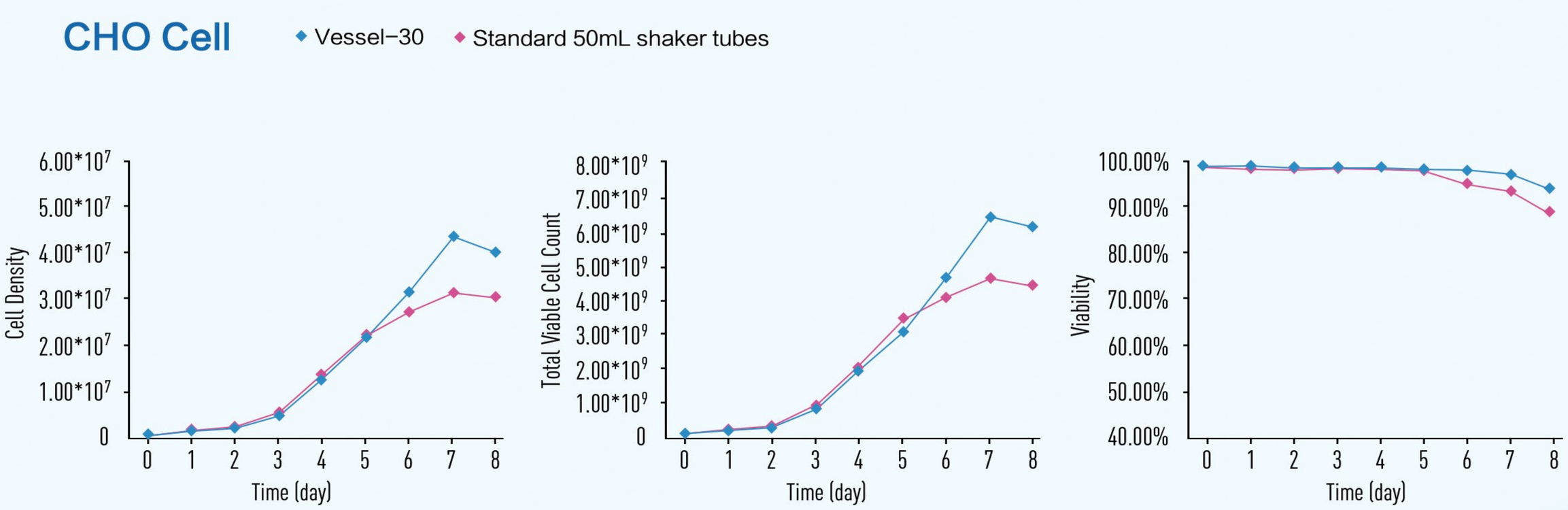
The recommended culture volume is 10−25mL and the inoculation density is ~5E+05 cells/mL.



Jurkat cells can also be cultured at high density in ImmunoVessel−30, reaching up to 1.6E+07 cells/mL.

03 For CHO cells cultured and expanded in ImmunoVessel−30, and 50mL shaker tubes

The recommended culture volume is 10−15mL, with an inoculation density of 3~5E+05 cells/mL. The shaking bed amplitude is 50mm, and the recommended speed is 120−150rpm.

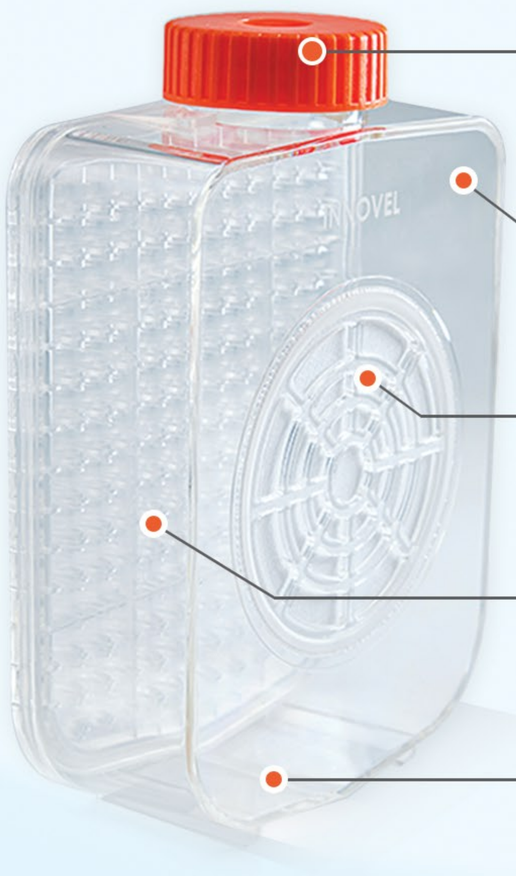


In ImmunoVessel−30:

CHO cell culture density can reach over 4E+07 cells/mL, significantly higher than in standard 50mL shaker tubes and comparable to the culture effect in shaker flasks.

ImmunoVessel-220 Flask

- Innovative patented double layered permeable membrane for superior gas exchange and maximum cell growth
- Compact design with 200mL volume to maximize incubator space and support ultra-high cell density cultures
- Biocompatible high-grade medical materials for transparency and superior results
- Individually packed and gamma sterilized



Micro injected reinforced cap for 100% tightness

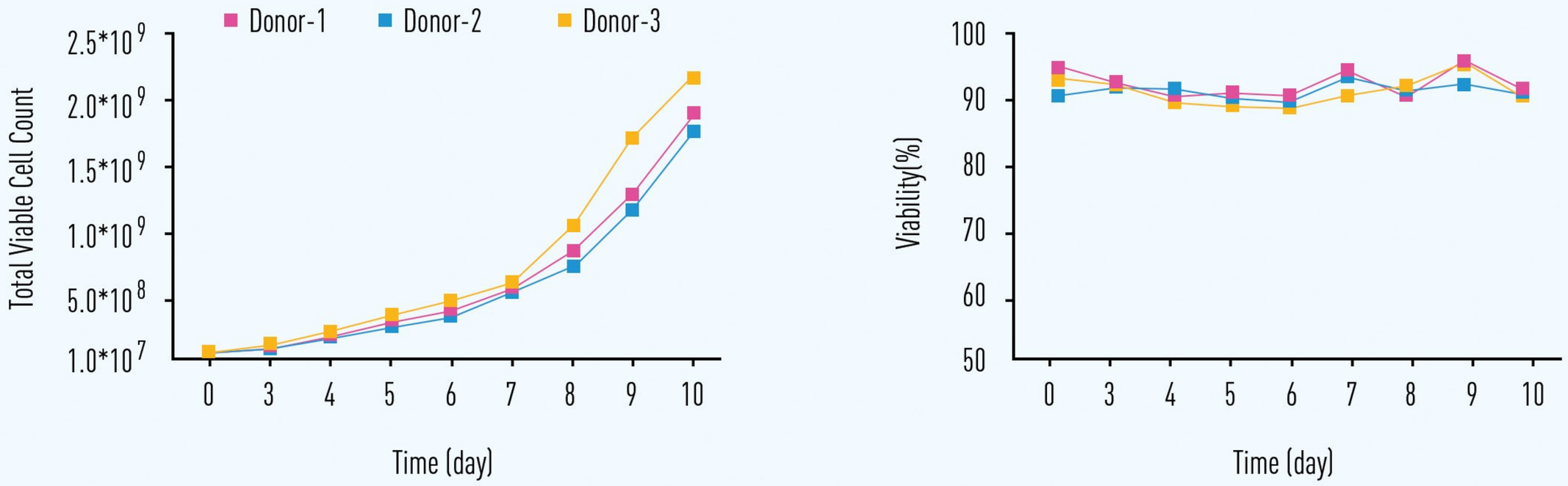
Internal body lining for high-performance

The unique multilayered membrane design

Honeycomb sublayer for superior cell adherence

Ergonomic design for maximum storing, stacking and transport

1st Expansion



2nd Expansion

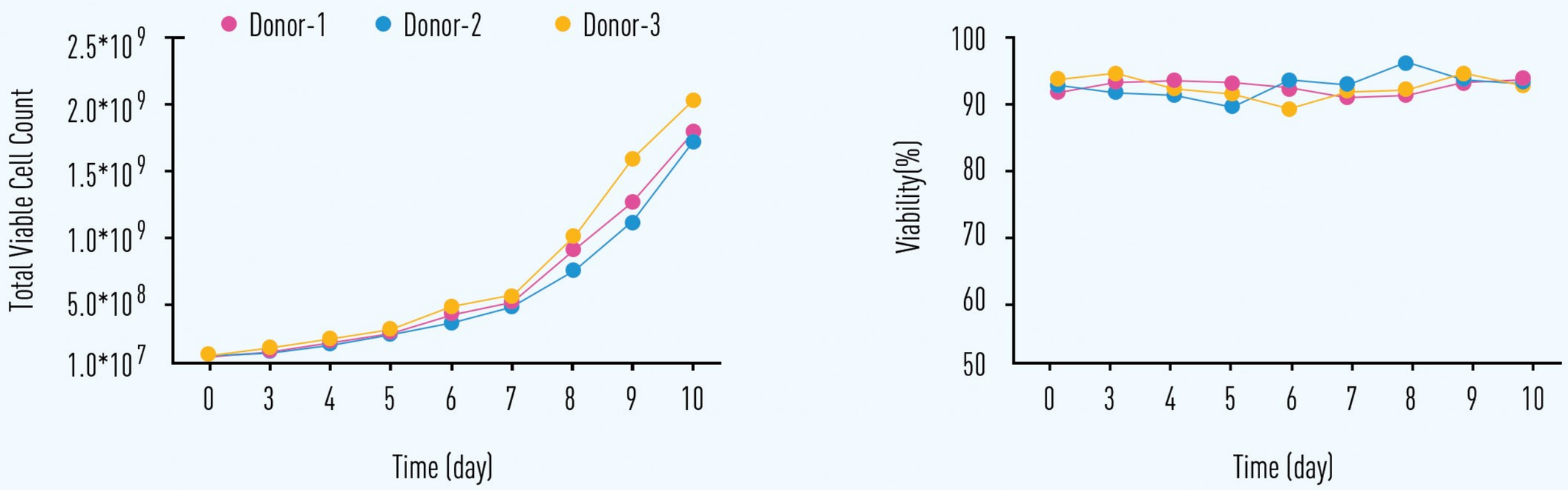


Figure 1: CAR−T Cell Expansion in ImmunoVessel

Expanding CAR−T cells from different donor sources in ImmunoVessel shows that within ten days of expansion, cells can proliferate more than 200 times in a highly active state!

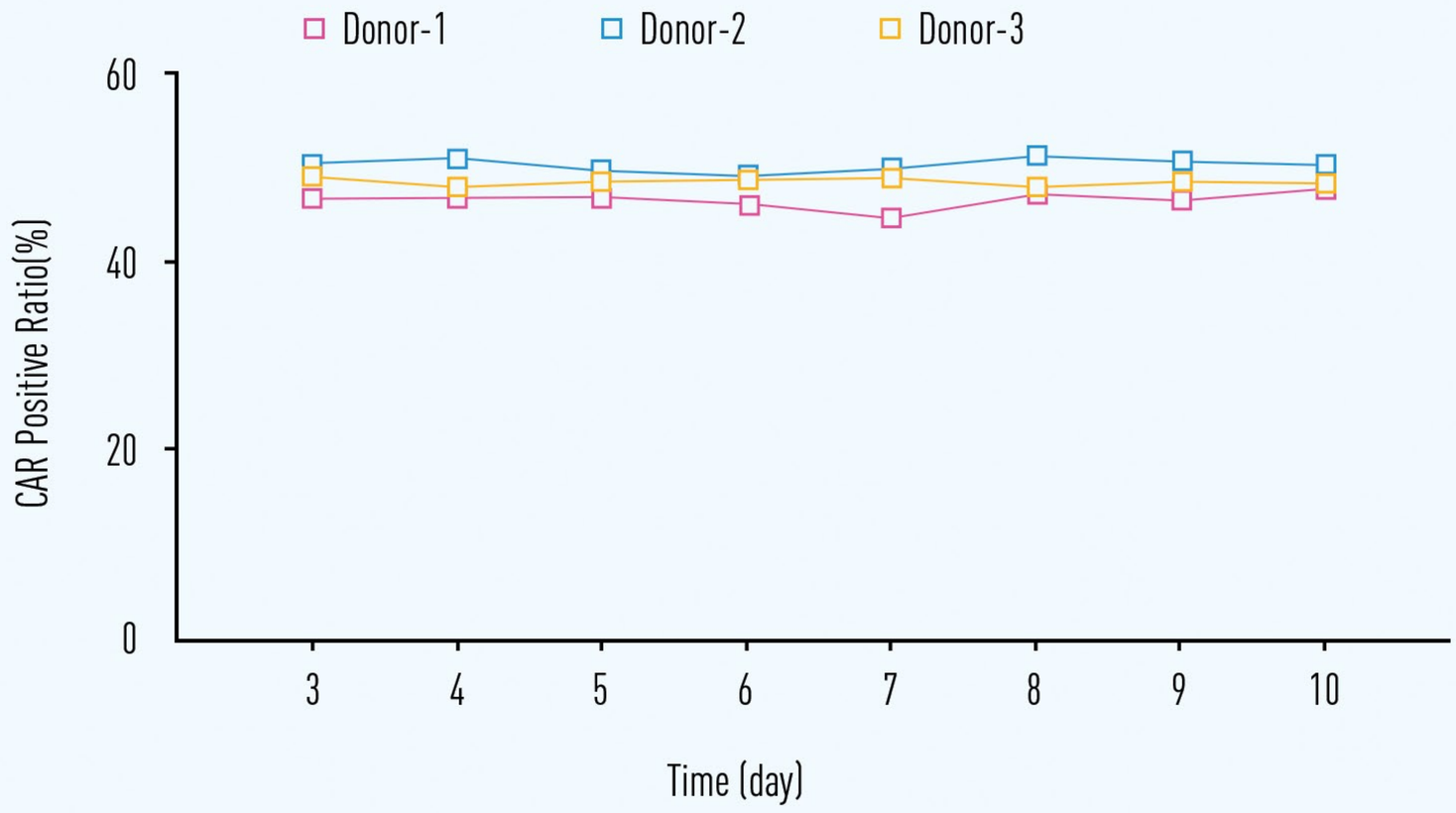


Figure 2: CAR−T Cells CAR Transduction Efficiency

During the expansion period, the positive rate of CAR molecules remains stable. CAR−T cells not only exhibit numerical superiority but also ensure high quality in growth.

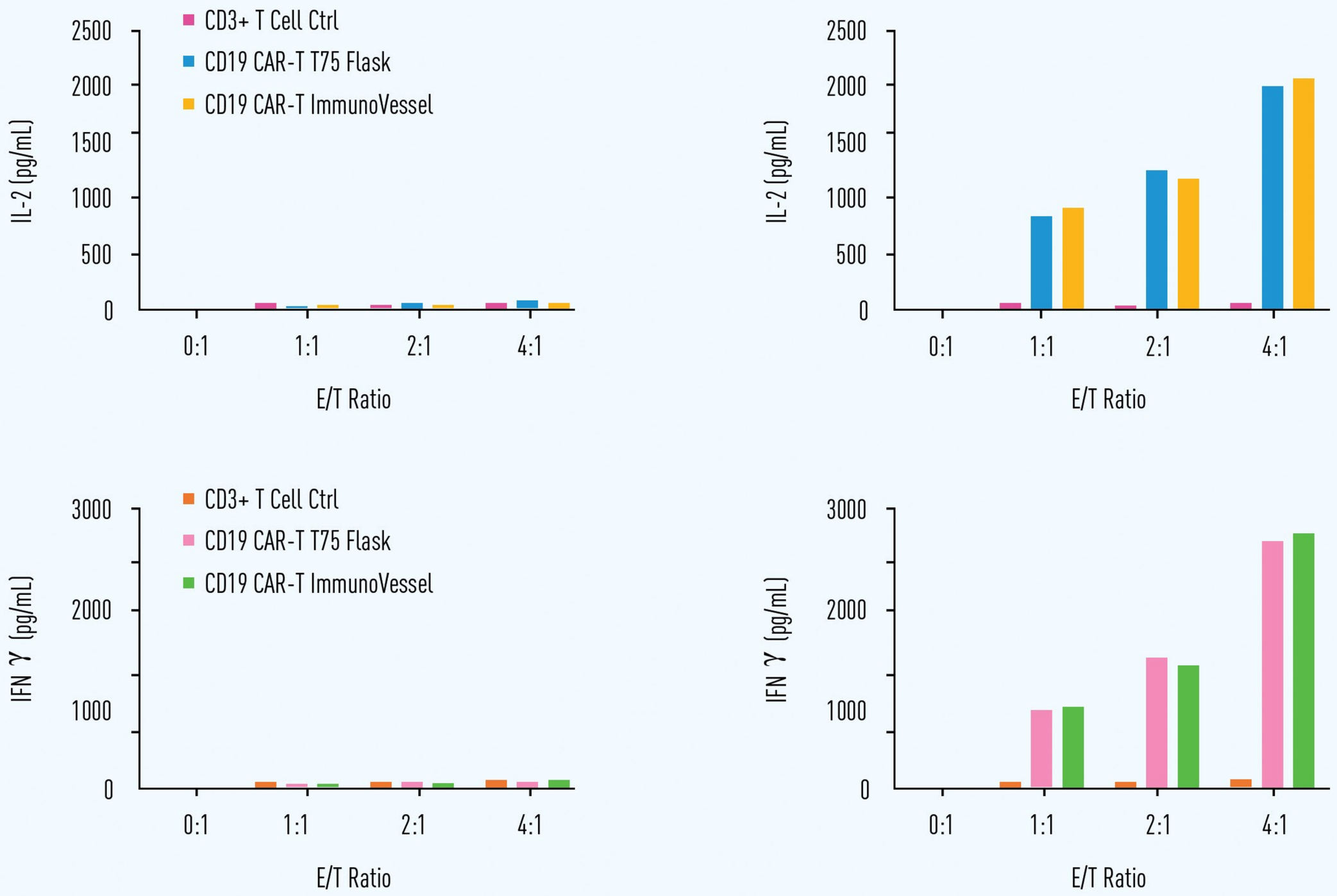


Figure 3: Cytokine Release Levels During CAR-T Cell Killing of Target Cells

When CAR-T cells are co-cultured with target cells, the levels of IL-2 and IFN γ release are comparable to traditional culture flasks.

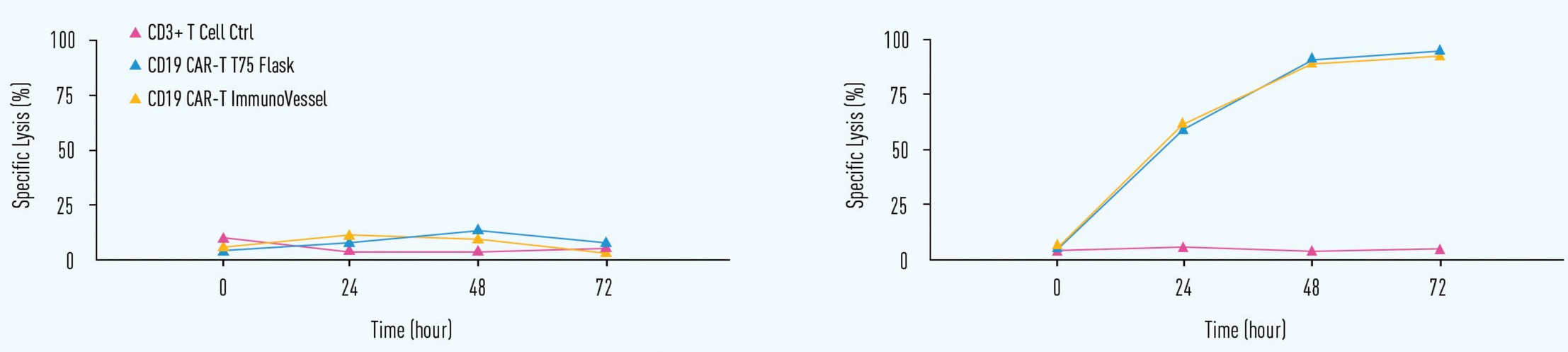


Figure 4: In Vitro Killing Level of CAR-T Cells Against Target Cells

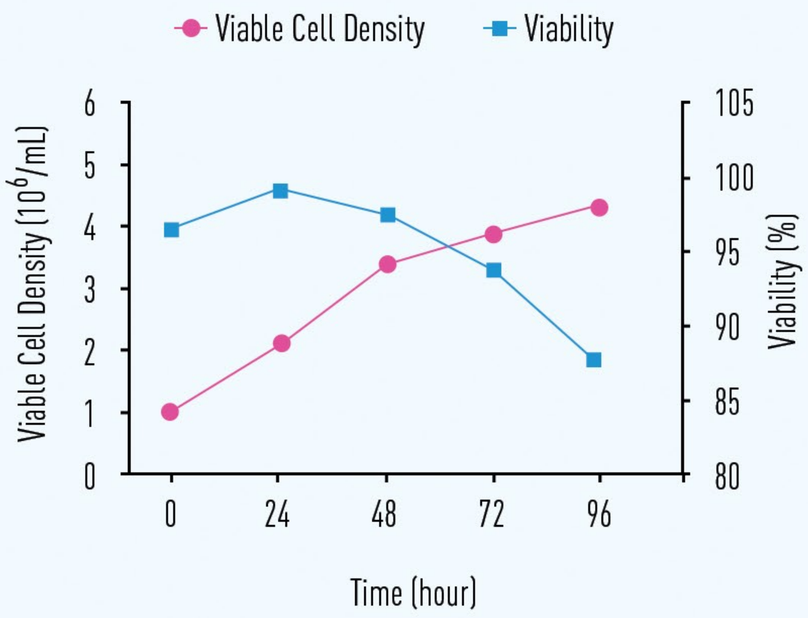
The in vitro killing level of CAR-T cells against target cells is comparable to traditional culture flasks.

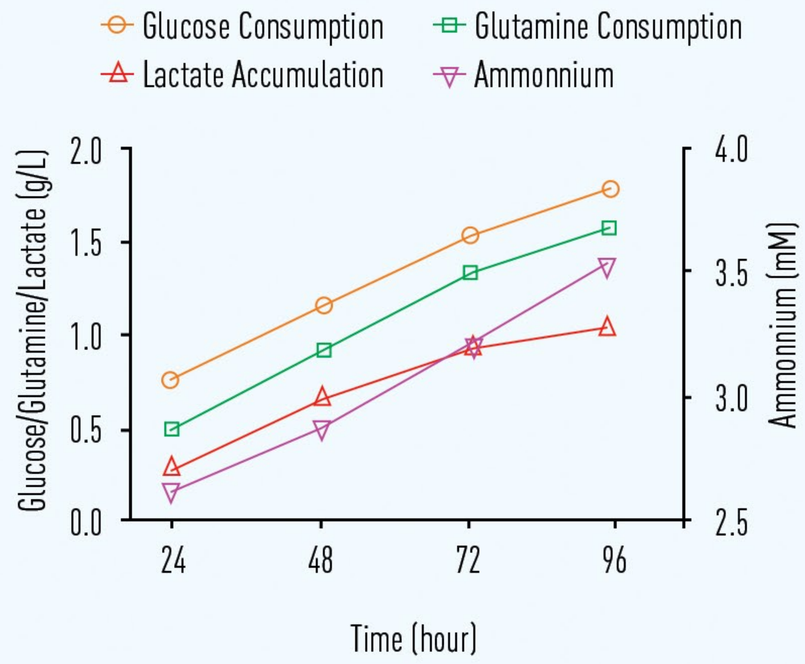
Other suspended cell lines (such as HEK293F, CHO, etc.) show continuous growth and maintain high cell viability.

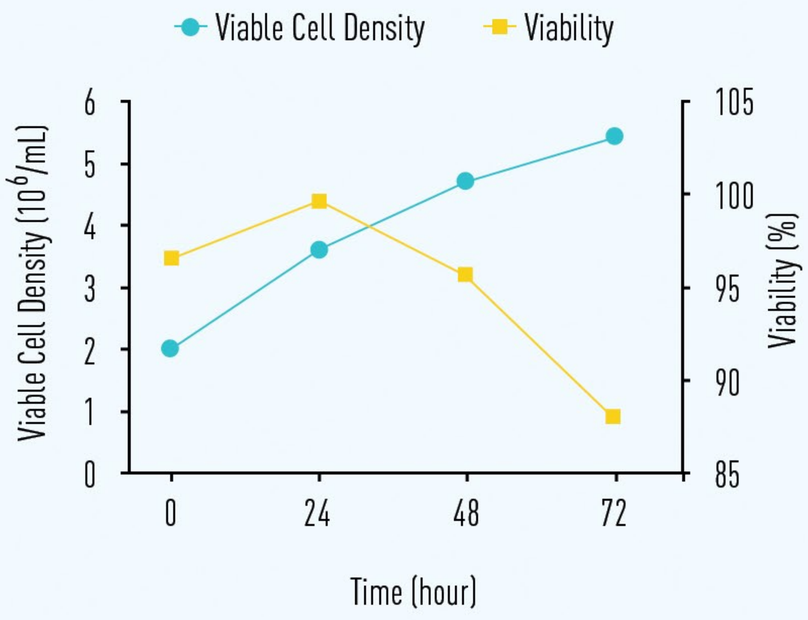
Cell lines like NK-92, Hybridoma, etc., can also be cultured in ImmunoVessel, maintaining high density.

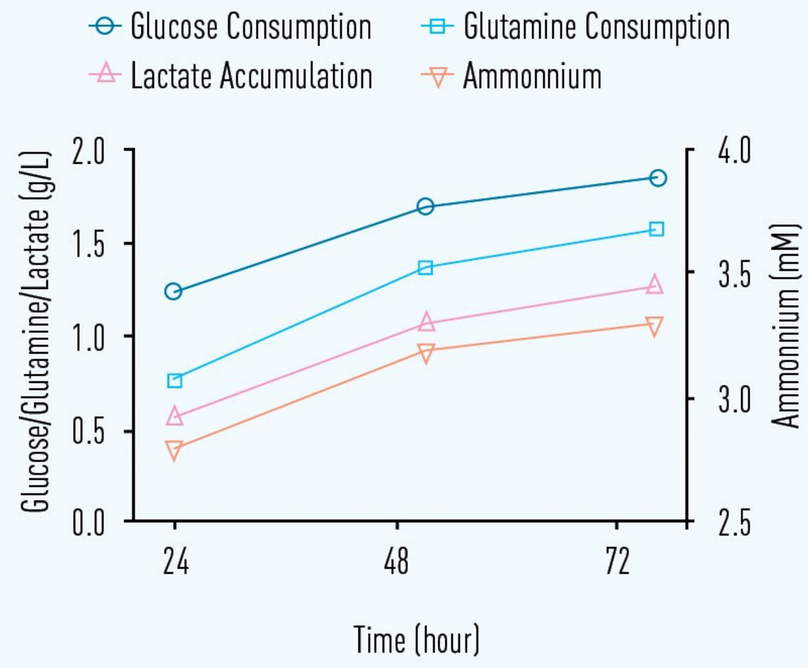
Velsson's Research on CAR-T Cell Culture in ImmunoVessel Bottles

Researchers at Velsson conducted a detailed biochemical parameter tracking analysis of the process of cultivating CAR-T cells in ImmunoVessel bottles. ImmunoVessel bottles have undergone various optimizations, especially in terms of breathability. The researchers aimed to provide users with a more reasonable reference by specifically analyzing metabolic indicators closely related to cell proliferation, such as the consumption rates of glucose and glutamine, as well as the accumulation of lactate and similar substances, to derive more detailed performance characteristics of the consumables.

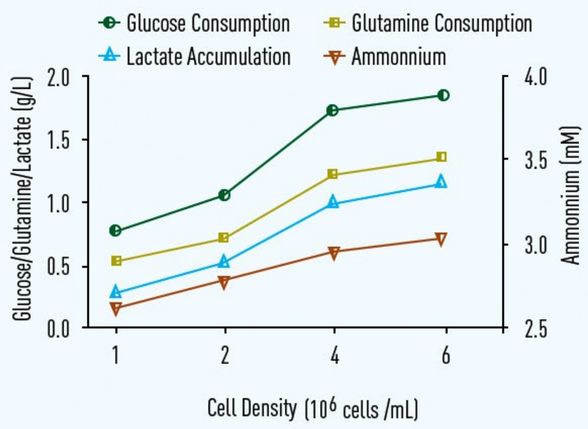




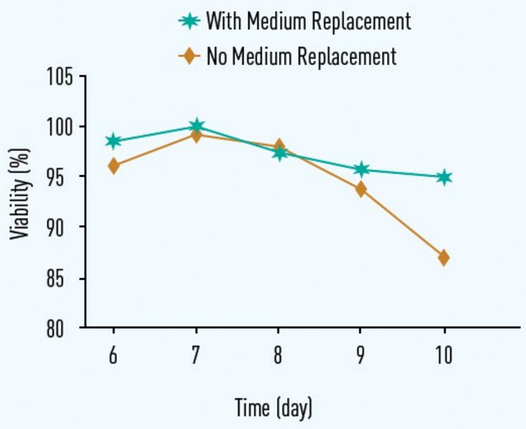


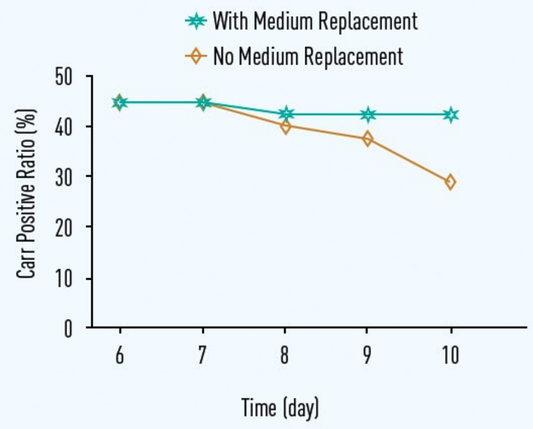


When seeding CAR-T cells at densities of 1E+06 cells/mL and 2E+06 cells/mL, without replenishing or changing the medium, the researchers continuously monitored the consumption of nutrients (glucose and glutamine) and the accumulation of metabolic waste (lactate and similar substances) during the cell growth process. The experiment revealed that over time, nutrients were gradually consumed, while metabolic byproducts continued to accumulate. At this point, the cell growth rate began to slow, and cell viability also showed a declining trend.



In ImmunoVessel bottles with different CAR-T cell seeding densities, the researchers detected the consumption rates of nutrients (glucose and glutamine) and the accumulationrates of metabolic waste (lactate andammonium) within 24 hours. The results indicated that at a seeding density of 4E+06 cells/mL after 24 hours of cell growth, the concentration of accumulated ammonium was close to 3mM. Additionally, the higher the cell density, faster the accumulation of ammonium and lactate.





After seeding CAR-T cells, prolonged periods without medium change resulted in the accumulation of metabolic waste. Significant decreases in both cell viability and the key indicator of CAR positivity were observed. Timely medium changes, introducing fresh culture medium while removing metabolic waste, were found to be beneficial for maintaining CAR positivity. Based on the analysis of multiple experimental results, it is recommended that when expanding CAR-T cells using ImmunoVessel bottles, daily medium changes are required once the cell density exceeds 3E+06 cells/mL to sustain rapid cell growth and ensure the quality of CAR-T cells.

Analysis of ImmunoVessel in CAR-T Cell Culture: Time and Density Effects

Researchers conducted an analysis of the correlation between the mentioned biochemical indicators and the growth time of cells, as well as the cell density, commonly known as time and dose effects in biological experiments.

The experiment revealed that when cultivating CAR-T cells in ImmunoVessel, the consumption of glucose and glutamine continuously increased with the extension of time. Simultaneously, lactate and similar substances continued to accumulate. Cells at a higher seeding density (2E+06 cells/mL) exhibited faster nutrient consumption and faster accumulation of metabolic waste compared to lower seeding density (1E+06 cells/mL). Additionally, an analysis of biochemical indicator changes within 24 hours under a gradient of cell seeding densities was conducted. The experiment showed that at cell densities higher than 4E+06 cells/mL, nutrient consumption and metabolic waste accumulation within 24 hours were very high, making it unsuitable for longer cell culture without medium change. For crucial cell culture experiments, such as CAR-T immunotherapy, it is recommended to change the medium when the cell density exceeds 2E+06 cells/mL in a single ImmunoVessel bottle. Fresh culture medium, combined with the excellent breathability of ImmunoVessel, is sufficient to support cell densities exceeding 1E+07 cells/mL in a fully liquid state (200mL), yielding a cell production of over 2E+09 cells.

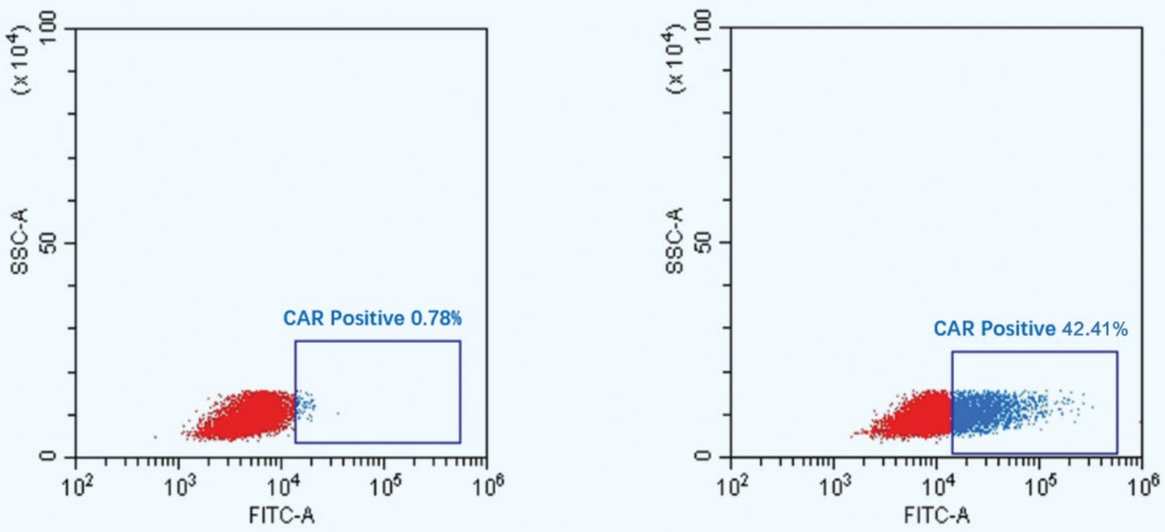
In the specific cell culture process of CAR-T cells, researchers unexpectedly found that prolonged periods without medium change, due to nutrient consumption and metabolic waste accumulation, deteriorated the cell survival environment. This led to a continuous decrease in the CAR positivity rate of CAR-T cells. The CAR positivity rate, as a core indicator in CAR-T cell therapy, has a decisive impact on treatment effectiveness. Therefore, ample nutrients, oxygen supply, and an appropriate growth environment are crucial for the production of CAR-T cells, creating greater value in therapy.

Velsson's Customer-Centric Approach

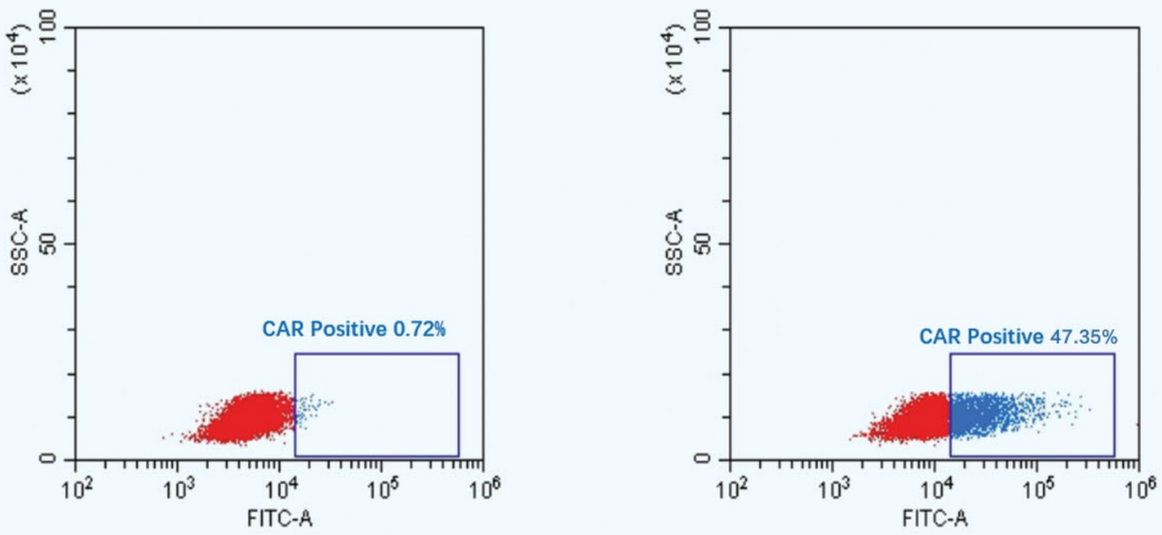
Velsson, with a customer-centric focus, aims to assist customers in cost-saving and more efficiently conducting production activities with the outstanding performance of its developed ImmunoVessel culture bottles. The goal is to create greater value for customers by providing efficient solutions for cell culture, particularly in processes like cell therapy production.

CAR-T preparation experiments with T cells from three different donors were utilized, resulting in a cell transduction efficiency ranging from 40% to 50%. The CAR-T cells exhibited rapid expansion and the capability for high-density cultivation in ImmunoVessel culture bottles.

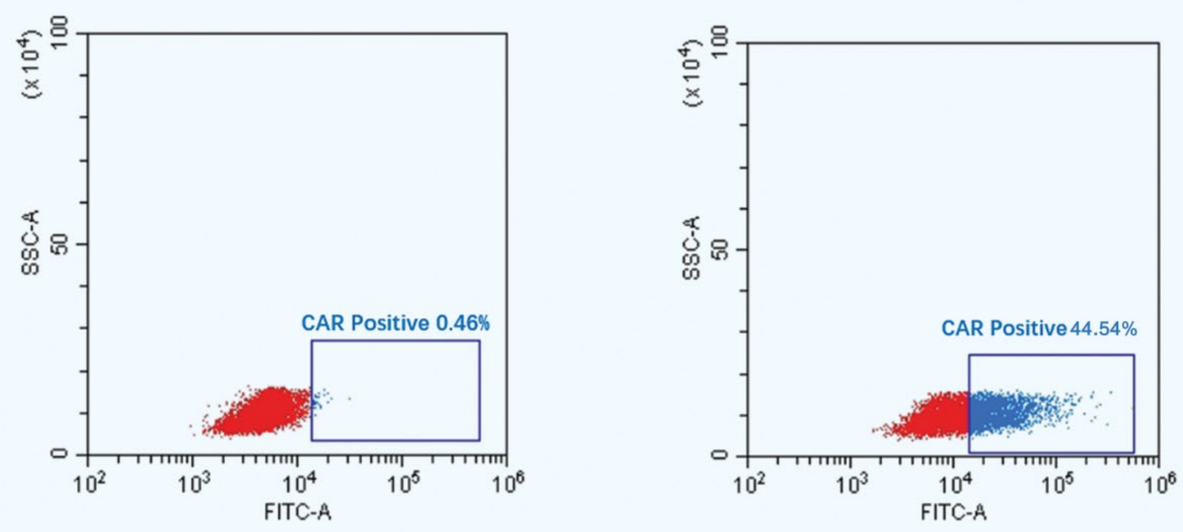
Donor-1



Donor-2

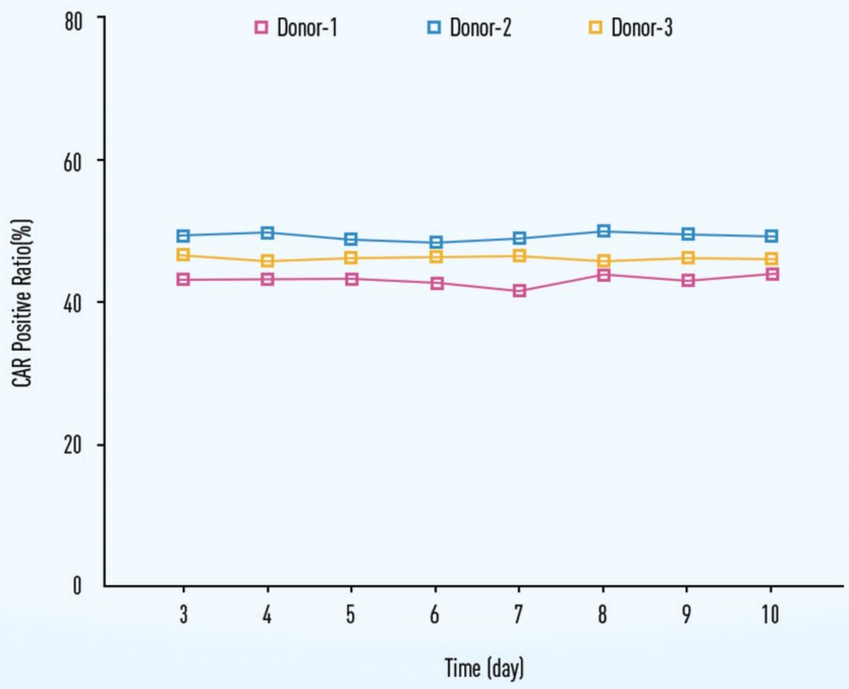


Donor-3



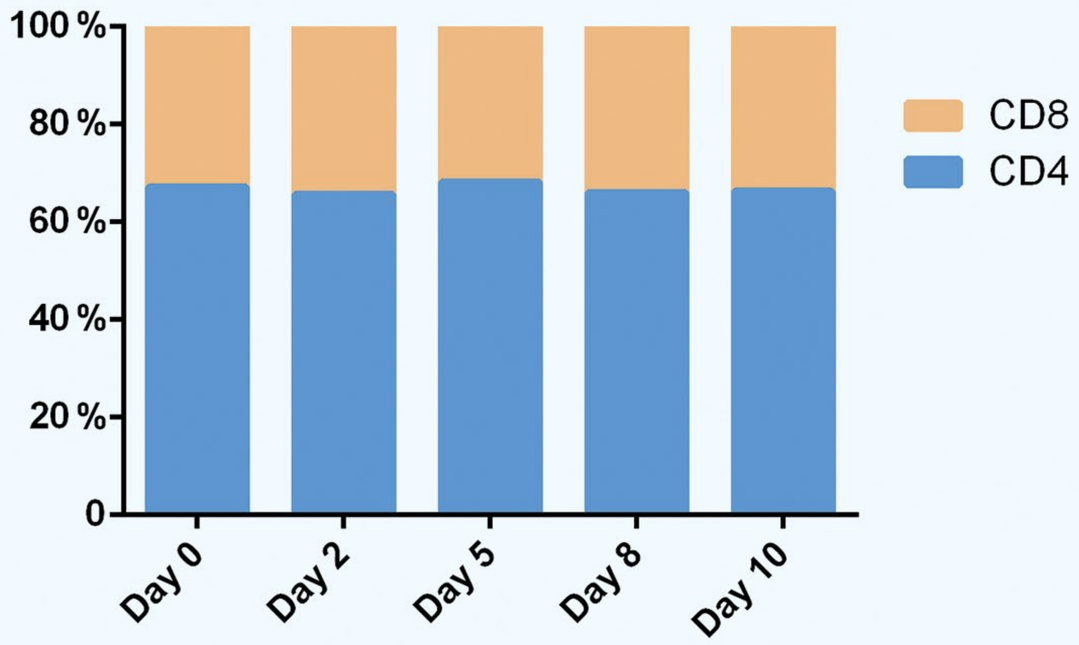
Transduction Efficiency of CD19 CAR-T Cells from Different Donors

T cells from three distinct donors (Donor 1, Donor 2, and Donor 3) were activated for 48 hours. They were then transduced with CD19 CAR lentivirus at an MOI of 20 to generate CAR-T cells. After 48 hours of transduction, CAR molecules were labeled with Protein L, and the transduction efficiency was measured using flow cytometry. The transduction efficiency is depicted in the figure.

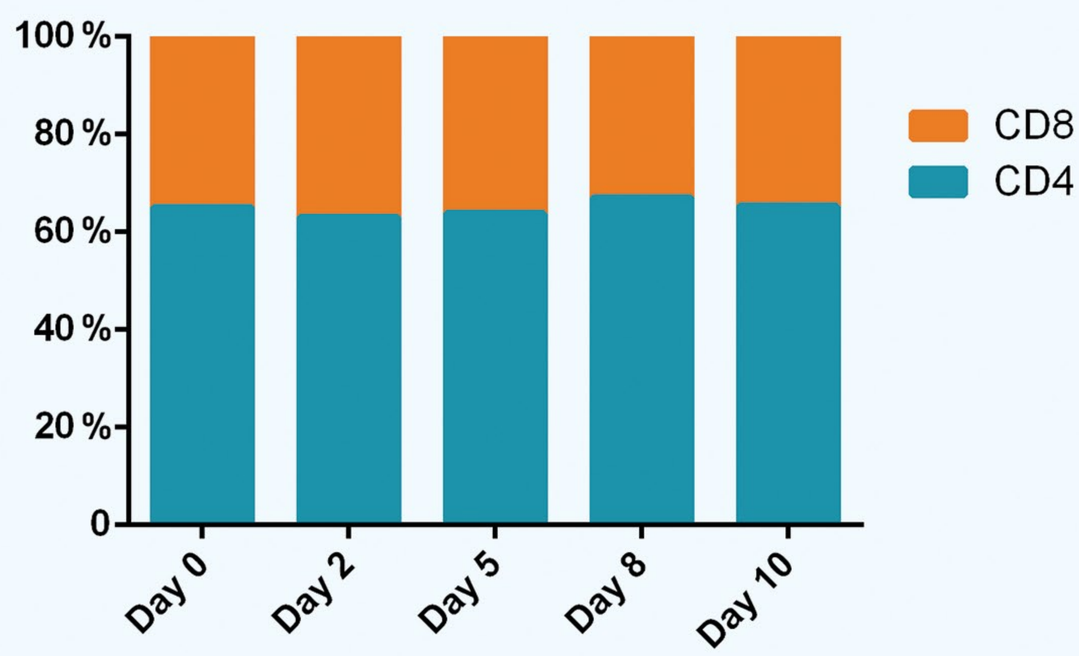


Throughout the expansion period, the positivity rate of CAR molecules remained constant. This indicates that CAR-T cells maintained both numerical superiority and quality in growth.

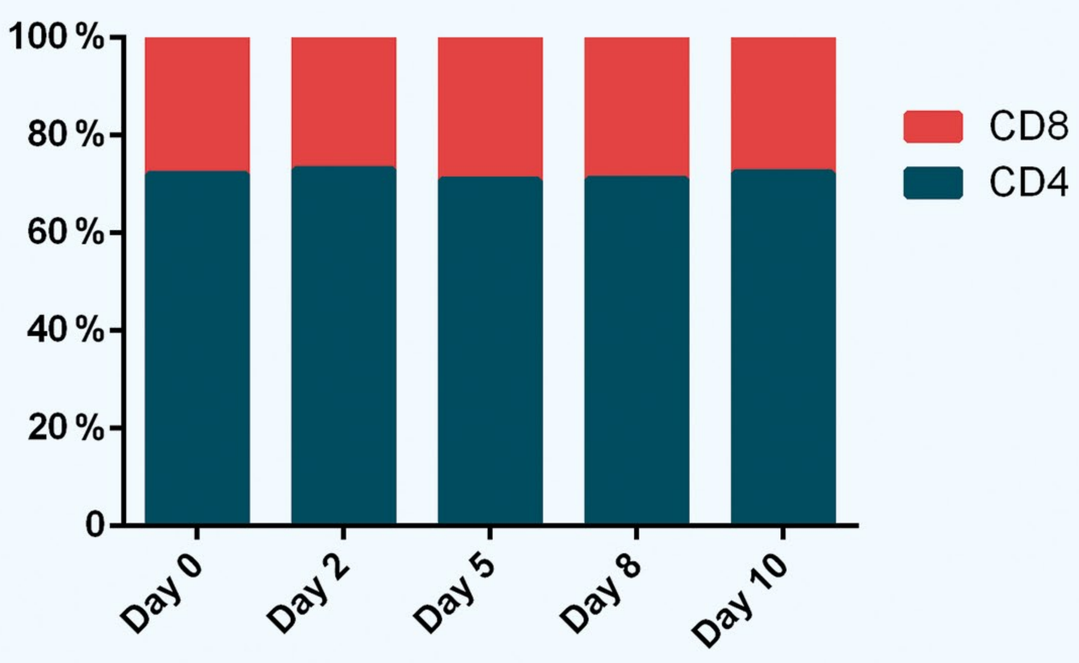
Donor-1



Donor-2



Donor-3



During the CAR-T cell preparation process, the ratio of CD4+ T-helper cells and CD8+ cytotoxic T cells remains stable.

Other suspended cells can also be cultured in ImmunoVessel. Some semi-adherent cell lines, such as CHO (Chinese Hamster Ovary) and HEK293F cells, can thrive in ImmunoVessel.

ImmunoVessel allows for tilted oscillation cultivation, maintaining high-density cell growth and continuous high cell viability.

01

More convenient to operate

1 220mL ImmunoVessel ≈ **8** T75 Flask ≈ **3** T175 Flask

01 T75 Flask
Recommended working volume of traditional T75 Flask culture bottle:15-25 mL.

02 Media consumed by other culture bottles

03 220mL ImmunoVessel
The volume of the culture bottle is 200mL, and the maximum culture volume is 180-200mL.

04 Media consumed by ImmunoVessel
Save 20%-30% Media.

05 T175 Flask
Recommended working volume of traditional T175 Flask culture bottle:35-55 mL.

During the operations, it is possible to significantly reduce workload, save time, and reduce the probability of cell contamination.

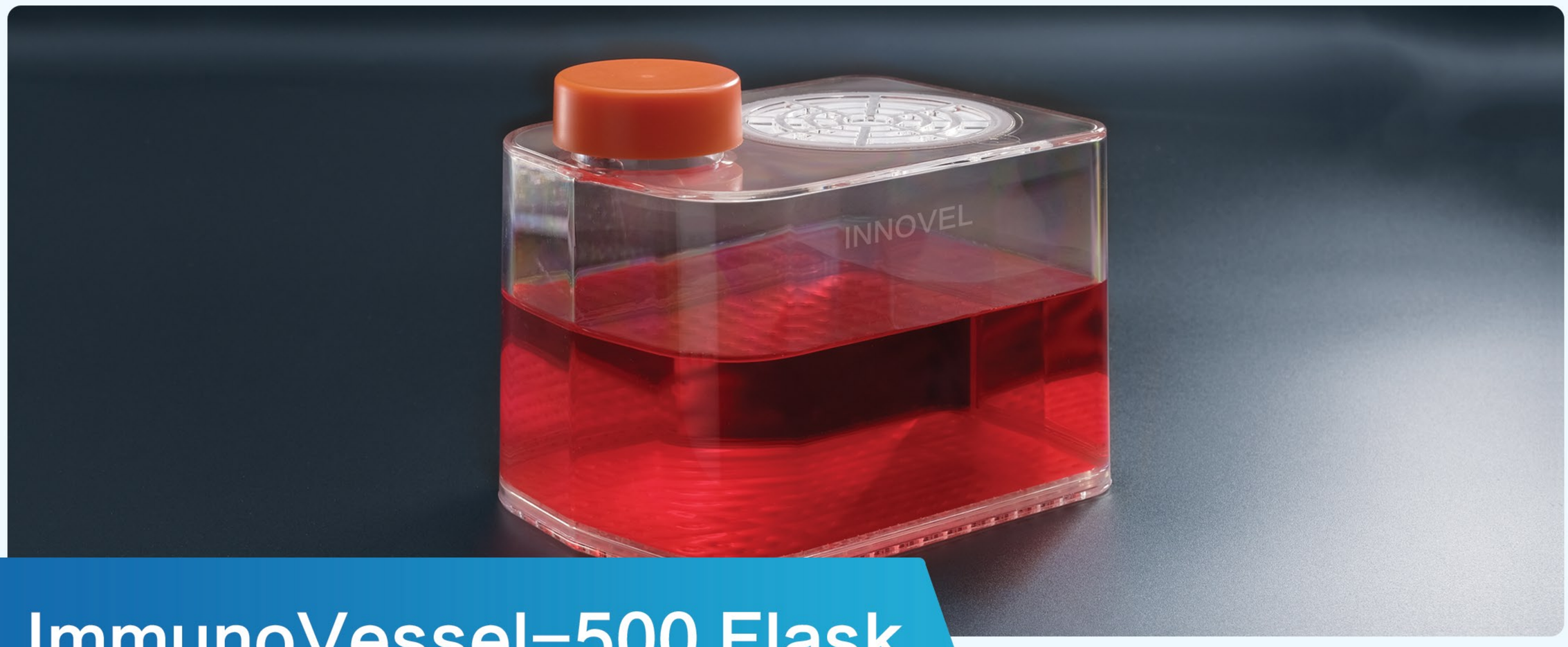
02

More cells with less bottles and incubators

ImmunoVessel Volume of a single flask: ~330 mm^3

T75 single flask volume: ~385 mm^3

T175 single flask volume: ~918 mm^3

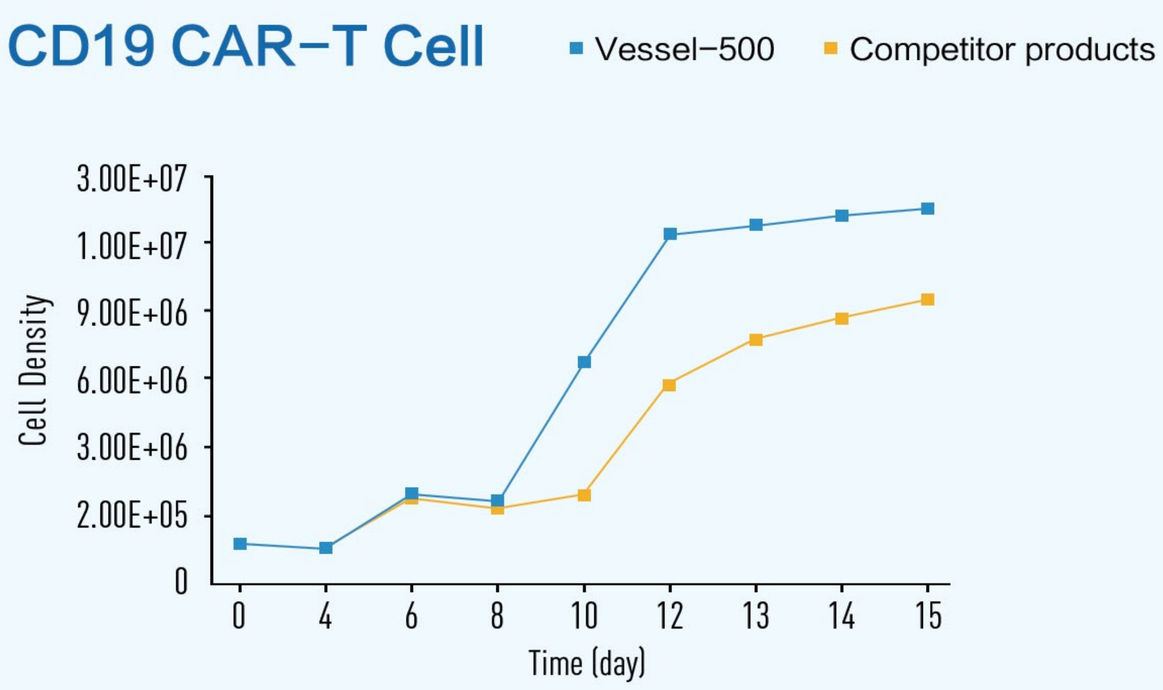


ImmunoVessel-500 Flask

It is recommended to use a cultivation volume of 50-400mL with a seeding density of ~5E+05 cells/mL.

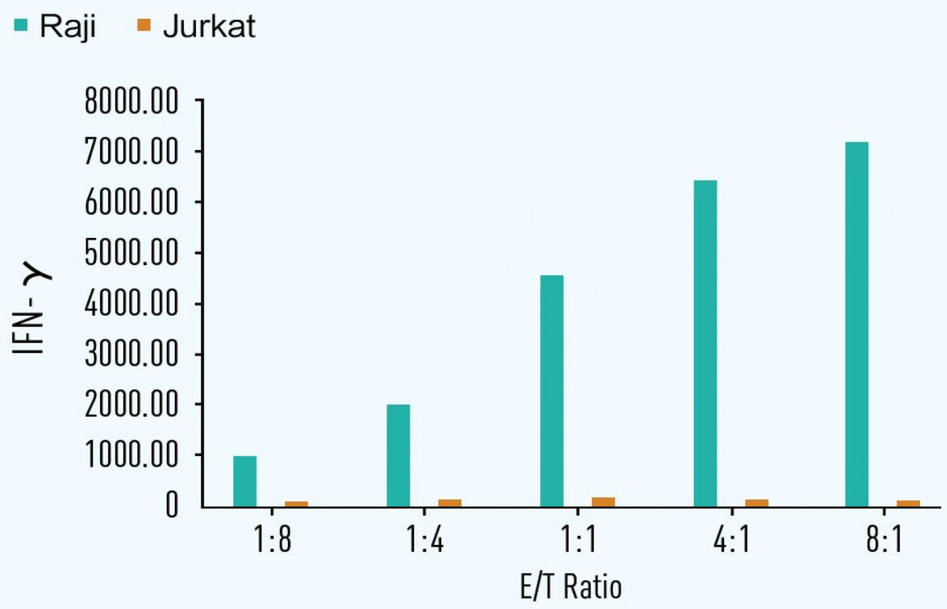
The design with an open top allows for media exchange without the need for centrifugation, avoiding potential cell damage and saving culture media. During cell harvest, a simple procedure involves settling the cells first, discarding the upper part of the culture supernatant, and then proceeding with centrifugation, making the process more straightforward and reducing the risk of contamination.

01 The cultivation and expansion of CD19 CAR-T cells were conducted in IMMUNOVESSEL-500 and compared to alternative methods.

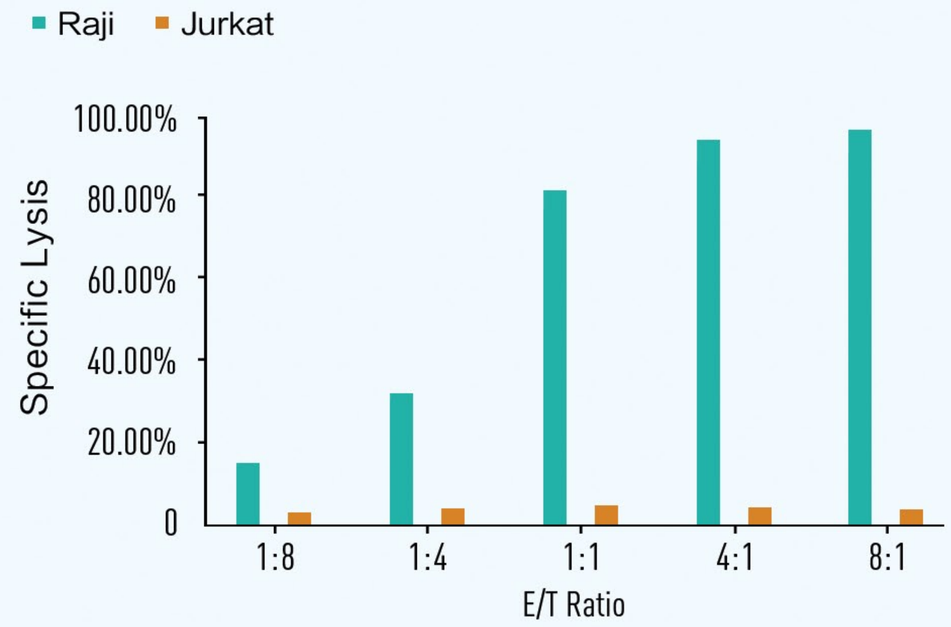


The cultivation performance of CD19 CAR-T cells in ImmunoVessel-500 bottles is equivalent to that in comparison products. After transfection for 8 days, the cell density exceeds 1.0E+07 cells/mL, with a viability exceeding 95%, and stable expression of CD19 CAR positivity.

CD19 CAR-T Cell Cytotoxicity/IFN-γ



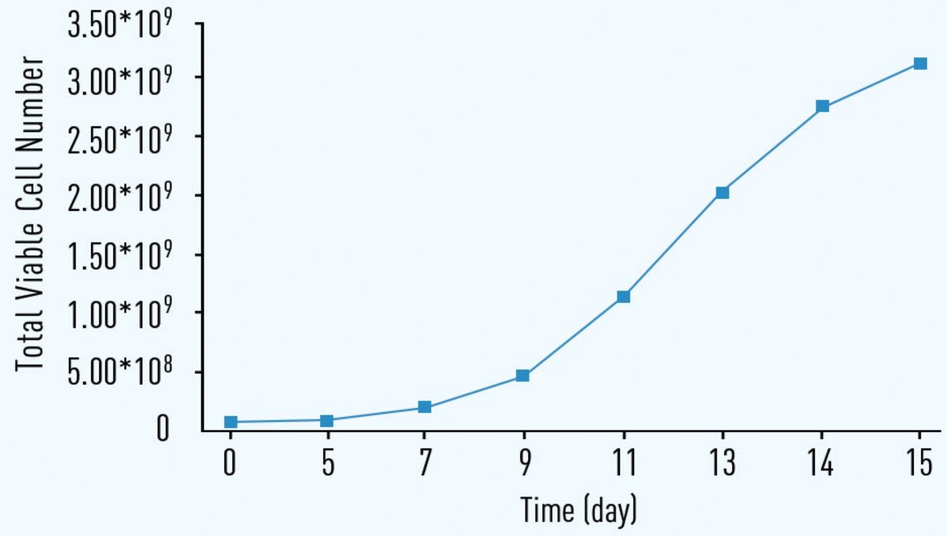
CD19 CAR-T Cell Cytotoxicity/Target Cell Lysis Rate

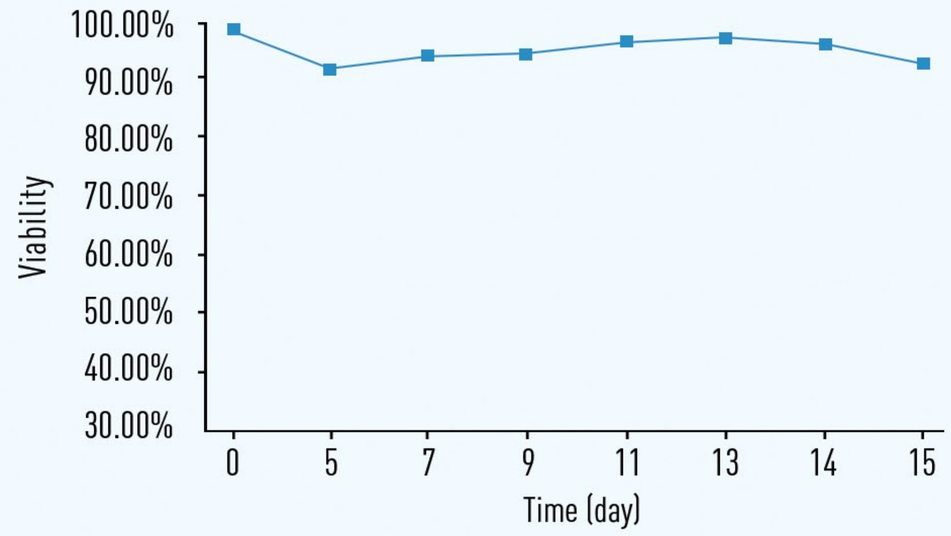


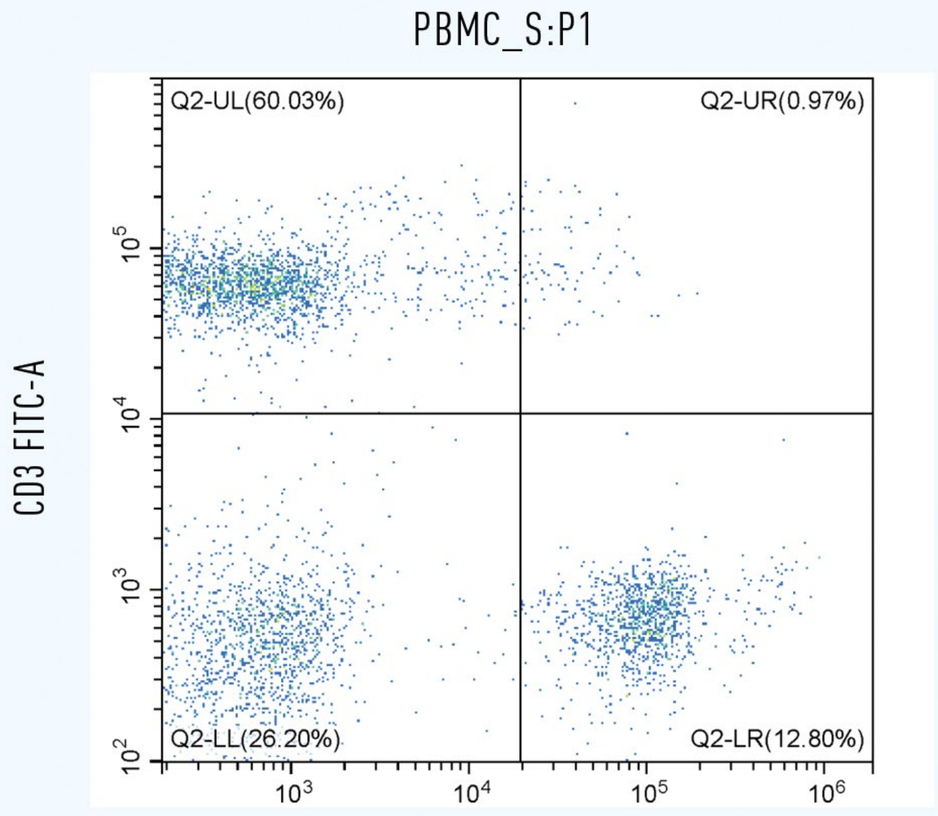
CD19 CAR-T cells cultured in ImmunoVessel-500 exhibit excellent killing effects against target cells.

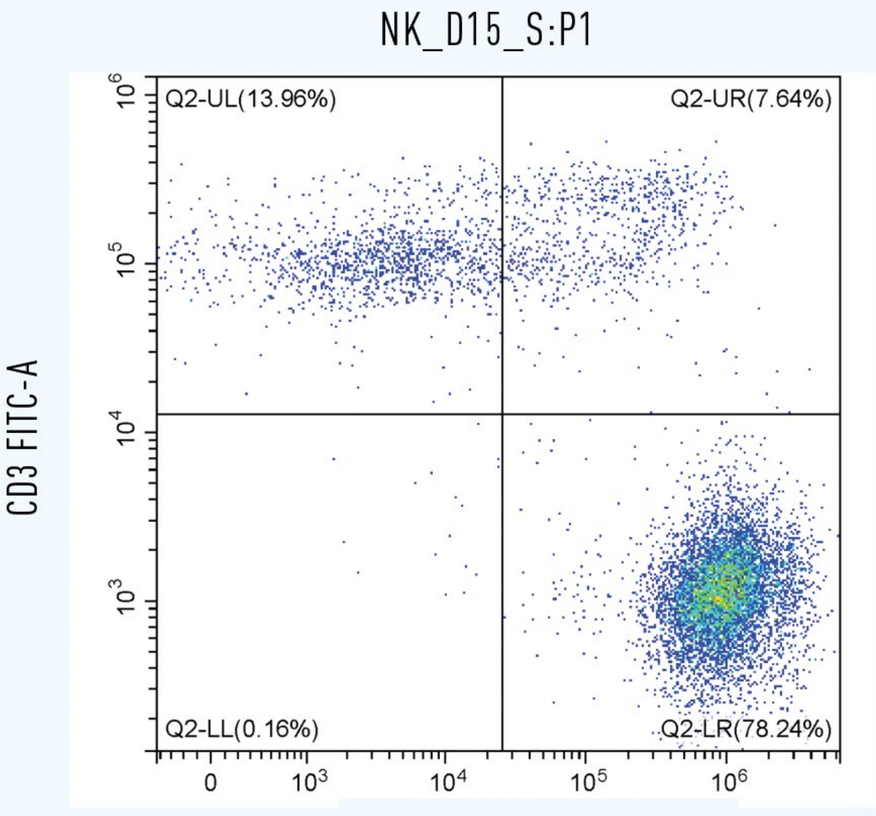
02 ImmunoVessel-500 culture bottles demonstrate outstanding performance in the cultivation and expansion of NK cells.

NK Cell









Starting with an initial PBMC count of 2E+07 cells, after activation induction, cultivation and expansion in ImmunoVessel-500 bottles allow the harvest of a total of 3E+09 cells from a single culture bottle, with a CD3-CD56+ ratio exceeding 75%.

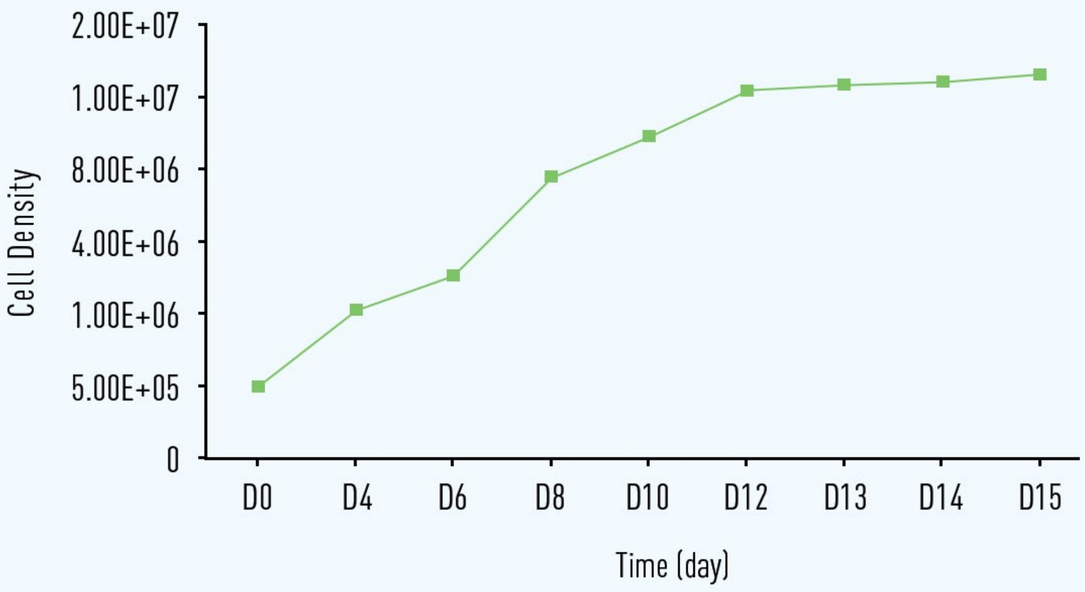
ImmunoVessel-1000 Flask

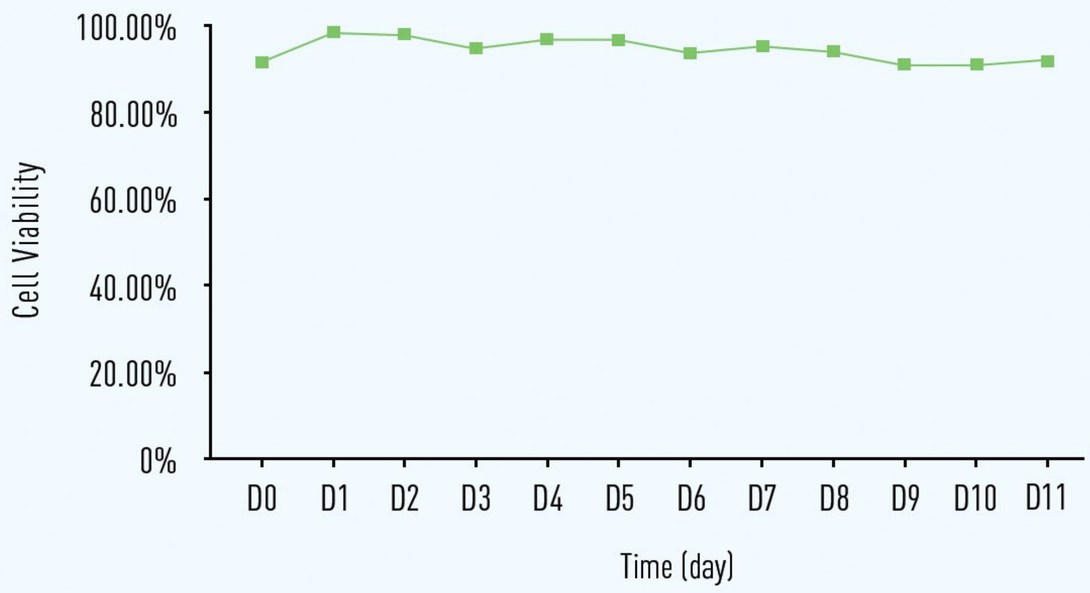
Recommendations for the culture include a volume range of 150-1,000mL and an inoculation density of ~5E+05 cells/mL.

The use of an open-mouth design allows for fluid exchange without centrifugation, preventing cell damage and conserving culture medium. During cell harvest, a simple procedure involves allowing the upper culture supernatant to settle before discarding, followed by centrifugation for cell collection, making the process more straightforward and reducing contamination risks.

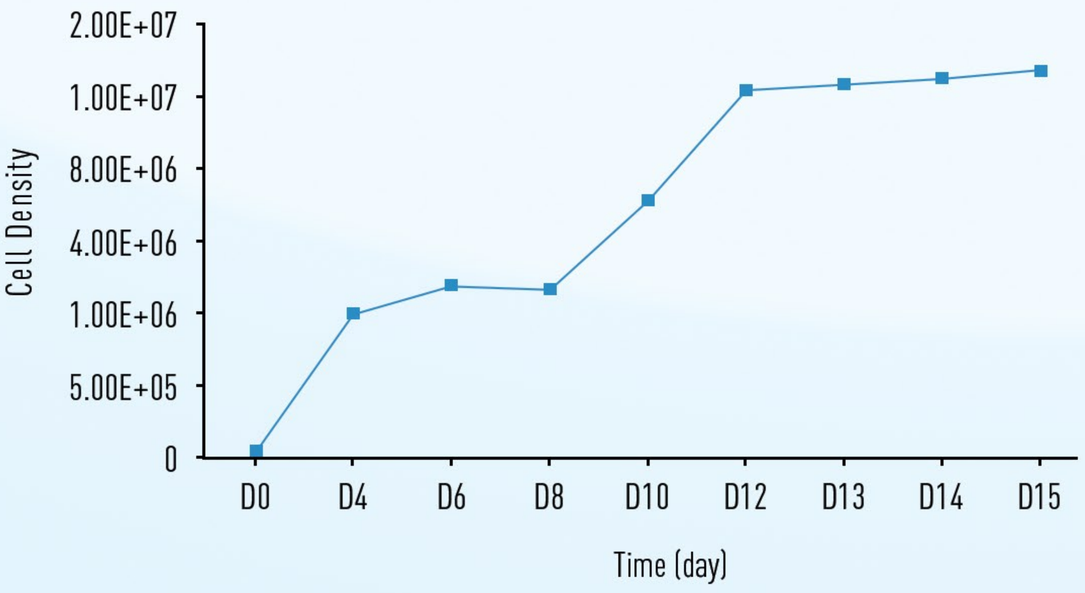
To meet the demand for a one-time culture with a substantial cell quantity, the ImmunoVessel-1000 culture bottle has a maximum volume of up to 1.2L, linearly increasing the number of harvested cells. Building upon the original design with top and bottom ventilation, the bottle's height and bottom surface area have been increased to enhance overall ventilation efficiency, providing a more conducive environment for cell growth.

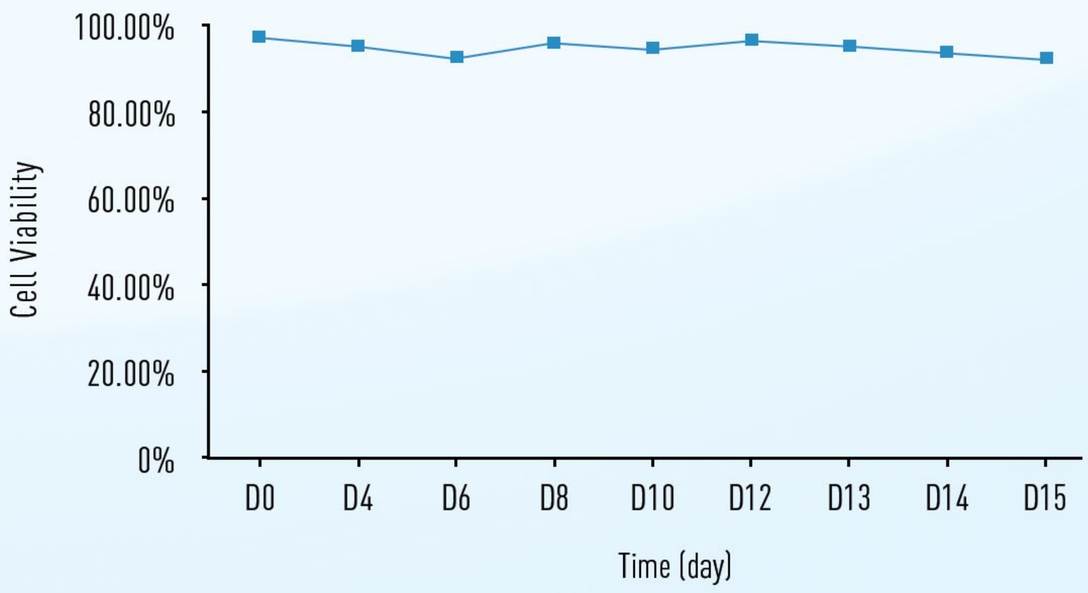
T Cell





CAR-T Cell





VELSSON
AN INNOVEL COMPANY

Customer Centric

Inspire Innovation

Integrity

Quality Excellence

Cost Effective

Collaborative Solutions

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