

# T cell Isolation using CD3/CD28 microbeads on MARS® Bar

The success of CAR T cell therapy in blood cancers highlights the need to optimize manufacturing for wider access, and efficient T cell isolation is crucial, impacting therapy effectiveness. Demand for better isolation methods is rising. CD3/CD28 microbeads are gaining attention for simplifying processes and enhancing workflow. They activate a wide range of T cells, promoting diverse responses. Our innovative approach uses Applied Cells Ingenuity<sup>TM</sup> 4.5  $\mu$ m beads, integrated into our automated MARS® Bar system, offering streamlined isolation with exceptional performance.

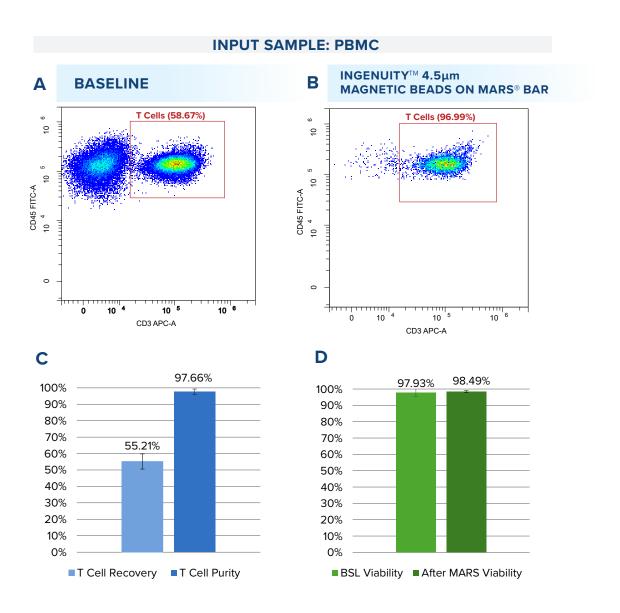


Figure 1. Efficient isolation of T cells from PBMC using the MARS® Bar platform, with high purity and no compromise in viability (A) PBMC prior to separation, and (B) T cells enriched using Ingenuity™ CD3/CD28 microbeads. (C,D) reflect the median performance of (B). (C) T cell positive selection with 1:1 bead to T cell ratio on MARS® Bar; average recovery of 55.21% T cells with an average purity of 97.66% from an initial average T cell purity of 57.89%. n= 4 PBMC samples. (D) Viability was not significantly affected after positive selection with Ingenuity™ CD3/CD28 beads on MARS® Bar (P<0.5).

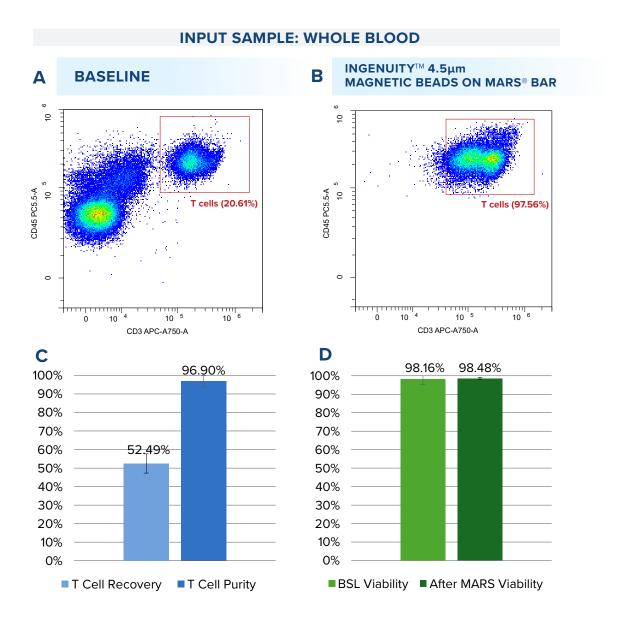
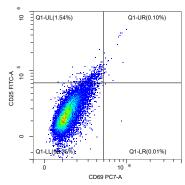


Figure 2. Purity of human T cells isolated using the MARS® Bar platform with no compromise in viability. (A) whole blood prior to separation, and (B) T cells enriched directly from whole blood sample using Ingenuity™ CD3/CD28 microbeads. (C,D) reflect the median performance of (B). (C) T cell positive selection using Ingenuity™ CD3/CD28 microbeads at a 1:1 bead to T cell ratio on MARS® Bar; average recovery of 52.49% T cells with an average purity of 96.90% from an initial average T cell purity of 25.10%. n= 3 healthy donor samples. (D) Viability was not significantly affected after positive selection with Ingenuity™ CD3/CD28 microbeads on MARS® Bar (P<0.5).

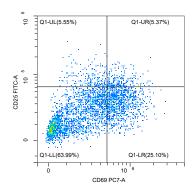
#### **Applied Cells Ingenuity<sup>™</sup> Reagents**

- 4.5µm size magnetic beads
- Detachable and removable by MARS Bar
- High stability, excellent separation efficiency of target cells
- Provide GMP grade products

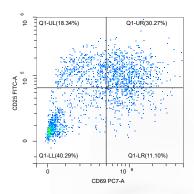
#### A - Ohr post enrichment



## B - 24hr post enrichment



#### C - 48hr post enrichment



#### D - 72hr post enrichment

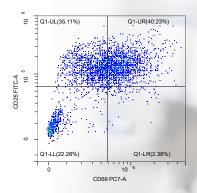


Figure 3 (left). Activation of enriched T cell using the MARS® Bar platform. 1E6 T cells isolated by Ingenuity™ CD3/CD28 microbeads at 1:1 bead to T cell ratio were cultured in per mL cell culture media for 3 days. Cells were stained with T cell activation markers CD25 and CD69 on (A) immediately post enrichment, (B) 24hr, (C) 48hr and (D) 72hr after enrichment. CD25 and CD69 expression are shown to be significantly increased with time.

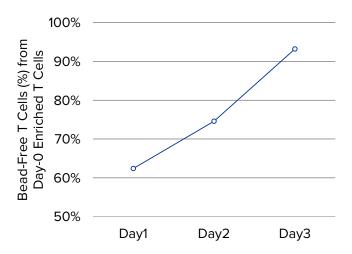


Figure 4. Depletion of Ingenuity™ CD3/CD28 microbeads and bead-bound cells using the MARS® Bar platform. Upon activation and culture of initially enriched T cells for 1~3 days, MARS® Bar platform was used to deplete mircobeads and cells bound with microbeads, at intervals of 24hr, 48hr and 72hr after start of culture. After microbead depletion, the initially enriched T cells from Day-0 yield recoveries of 62.4%, 74.6%, and 93.2% of bead-free T cells.

### About MARS® Bar

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#### MARS® Bar Cell Separation Platform

The MARS® Bar Magnetic Separation Platform is a closed and automated isolation for cell therapy development and manufacturing. Together with fit-for-purpose consumables, it delivers high cell purity, recovery, and viability.

Designed to be used with magnetic beads, MARS® Bar Magnetic Separation Platform uses column-free technology performs in-flow separation at high flow rate to achieve exceptional cell recovery, purity, a wide range of reaction volumes while maintaining cell viability. Together with process flexibility, sterile single-use consumables, and software with 21 CFR Part 11 compliance, this system is designed to help you easily scale from development to clinical and commercial manufacturing.

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