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ATRACT™
T-Cell Activation Kit

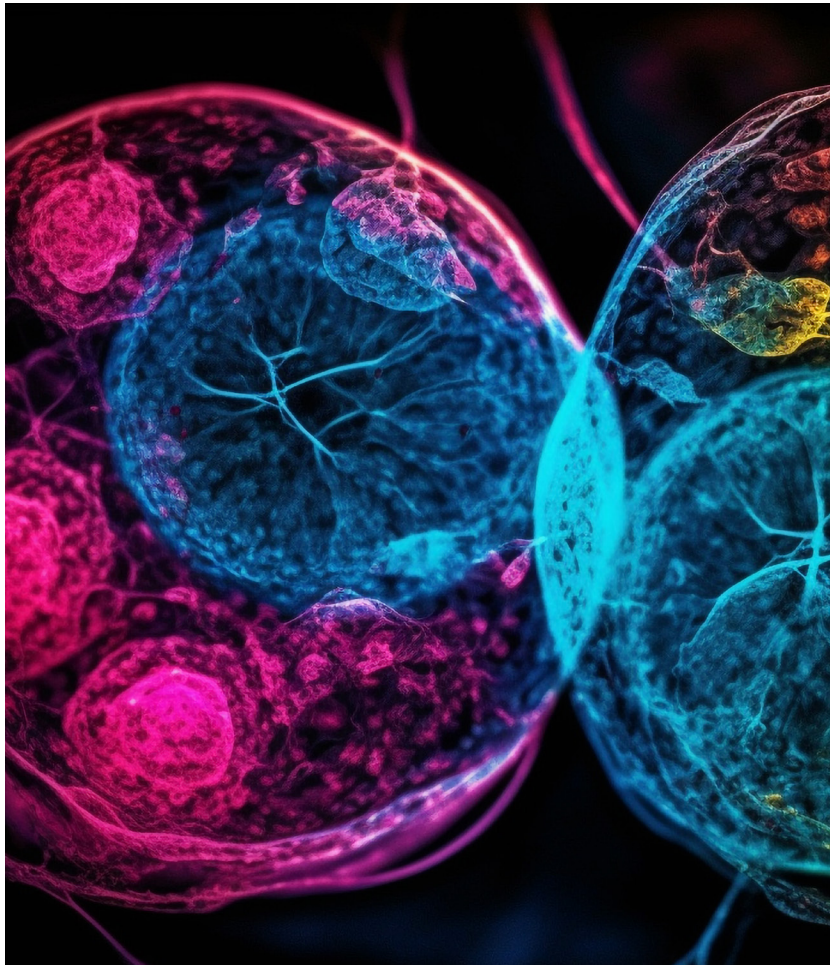
PRODUCT BROCHURE

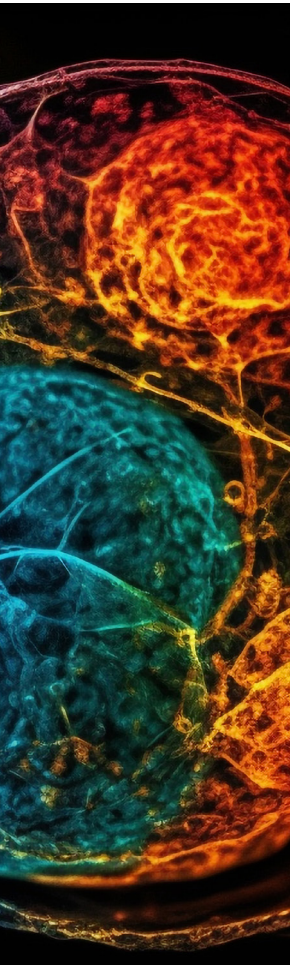
Bio-ReCell®

Table of Contents

OVERVIEW

Activation of T-cells is a crucial step in the immune response. Aberrant T-cell activation or deactivation plays a key role in infections, cancer, and autoimmune disorders. As such, this biological process is relevant to many clinical and research fields. To study factors that affect T-cell activation, researchers can utilize T-cell activation kits like the **Atract™ Kit** from **Bio-ReCell**, which allows them to test the effects of drugs or other factors in T-cell activation.





The **Atract™ Kit** product family contains two versions of biocompatible composites of irreversibly bound antibodies. Each offers specific advantages that can fulfill different customer needs. The **Atract™ Kit V1** is designed for optimal activation, providing a unique opportunity to fine-tune activity by adjusting the CD3 to CD28 bead ratio. The **Atract™ Kit V2** is optimized for superior proliferation and user-friendly operation. The reagents are designed to mimic antigen-presenting cells' role and activate naive T-cells derived from peripheral blood mononuclear cells (PBMCs) or purified T-cells. T-cell expansion is achieved through cultivation in a medium with the addition of interleukin 2 (IL-2) for up to 14 days. The **Atract™ Kit** reagents cannot be phagocytized. Furthermore, no antibodies are left in the culture once the reagents are removed. The reagents are compatible with conventional culture flasks, bags, and plates.

The performance of the **Atract™ Kit** products has been demonstrated through an array of T-cell activation assays, such as monitoring CD69 and CD25 expression. We compared the **Atract™ Kit V1** and **V2** to the leading market competitors and results show that both **Atract™ Kit** products match and even outperform the competition in crucial parameters.

Atract™ Kit V1

KEY FINDINGS

EXPERIMENTAL DESIGN: 12-WELL PLATE

Activation and expansion with **Atract™ Kit V1** is comparable to commercial kits regarding fold expansion, viability, activation markers, exhaustion markers, and T-cell subpopulations. After activation and reactivation, TNF-alfa and IFN-gamma levels were comparable to commercial kits. A lower CD4:CD8 ratio was observed with **Atract™ Kit V1** and MACSiBead™ Particles compared to TransAct™ and Dynabeads™.

2 days	**Atract™ Kit V1**	MACSiBeads™	Dynabeads™	TransAct™
CD69+ (%)	76	60	34	69
CD25+ (%)	88	82	22	87
Viability (%)	96	94	97	97

Key readouts of T-cell activation comparing **Atract™ Kit V1** to competitors 2 days post activation.

10 days	**Atract™ Kit V1**	MACSiBeads™	Dynabeads™	TransAct™
CD69+ (%)	12	6	25	9
CD25+ (%)	25	21	37	36
Fold expansion	1531	1415	11	2104
Viability (%)	97	96	83	97
CD4+ (%)	19	10	28	53
CD8+ (%)	64	83	58	43
Tcm (%)	62	51	48	43

Key readouts of T-cell expansion comparing **Atract™ Kit V1** to competitors 10 days post activation.

KEY FINDINGS

EXPERIMENTAL DESIGN: G-REX PLATFORM

The data comparing the **Atract™ Kit V1**, **Atract™ Kit V2**, and TransAct™ over 9 days shows that all three kits perform similarly in terms of overall cell expansion and viability, with **Atract™ Kit V2** showing the highest viability and cell count. Activation markers, such as CD69 and CD25, are relatively consistent, although TransAct™ displays a lower level of CD69 expression. Regarding T-cell subpopulations, **Atract™ Kit** products tend to favor CD8+ T cells, while TransAct™ results in a higher CD4+ percentage and central memory T-cell (Tcm) expansion.

9 days	**Atract™ Kit V1**	**Atract™ Kit V2**	TransAct™
CD69+ (%)	11	12	9
CD25+ (%)	82	72	85
Cell number (Million)	316	329	313
Viability (%)	84	89	86
CD4+ (%)	32	31	55
CD8+ (%)	56	57	43
Tcm (%)	46	40	61

Key readouts of T-cell expansion comparing **Atract™ Kit** to competitors 9 days post activation.

RESULTS BREAKDOWN

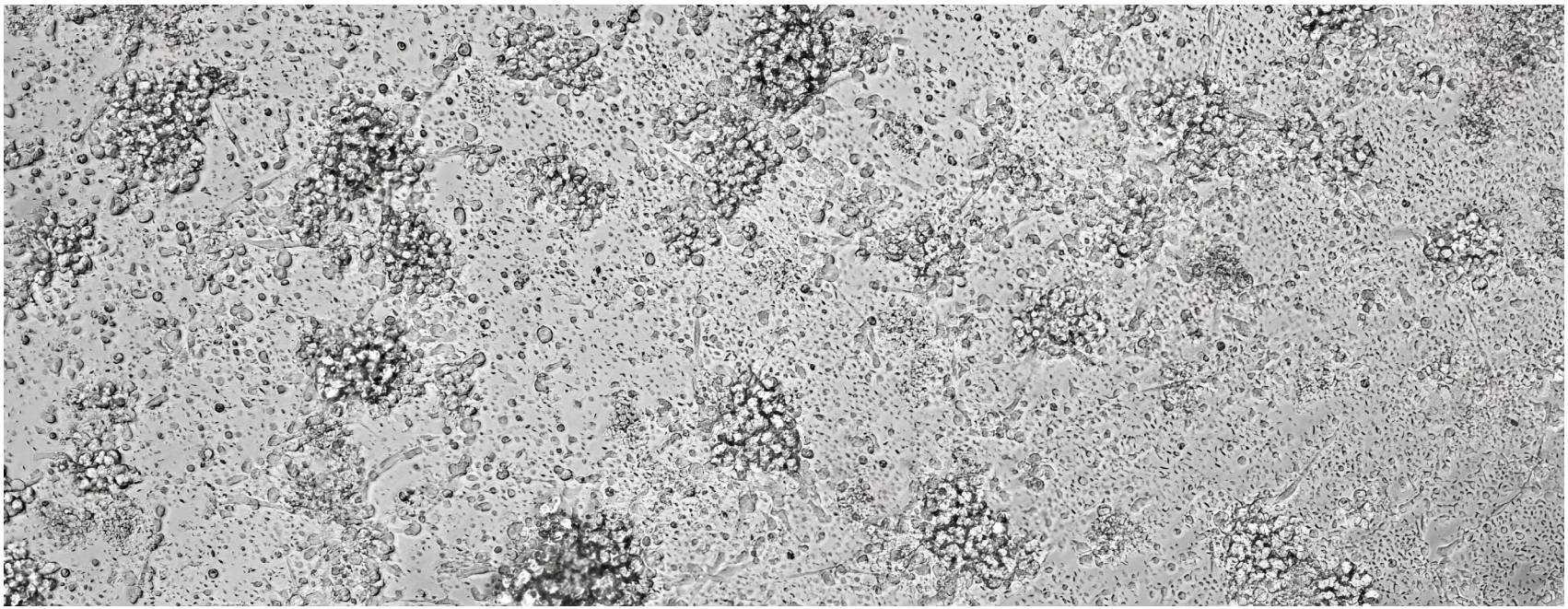
Experimental Design: 12-well Plate

We conducted small-scale experiments on 12-well plates to compare the **Atract™ Kit V1** with three commercial T-cell activation kits:

- Thermo Fisher CTS™ Dynabeads™ CD3/CD28,
- Miltenyi T Cell Activation/Expansion Kit (containing MACSiBead™ Particles),
- Miltenyi T Cell TransAct™.

Method:

Step	Time	Description
Medium Preparation	Day 0	CTS AIM-V medium was mixed with 10% FBS, PenStrep with antimycotics, and 200 U/mL IL-2.
Cell Preparation	Day 0	PBMCs from 3 healthy donors were isolated and diluted to 6 M cells/mL.
Atract Preparation	Day 0	The selected quantities of **Atract™** CD3 and **Atract™** CD28 reagent were mixed, washed in 1 mL of medium, and put into 0.5 mL of medium. For TransAct, 10 µL of TransAct reagent was mixed with 0.5 mL of activation medium.
Activation Setup	Day 0	0.5 mL of cells and 0.5 mL of activation reagent were mixed and pipetted onto a 12-well plate. Culture plates were transferred to the incubator (37 °C, 5% CO_2).
Expansion	Day 2	**Atract™ Kit V1** reagent was removed.
Expansion	Day 2-10	Cells were split daily and seeded at a $0.5x10^6$ cells/mL density.
Flow Cytometry Analysis	Day 2, 4, 7, 9, 10	Multicolor flow cytometry was performed for marker analysis. Supernatants were sampled on days 3 and 10 (24h after reactivation) and analyzed with an ELISA assay for IFN-gamma and TNF-alpha.

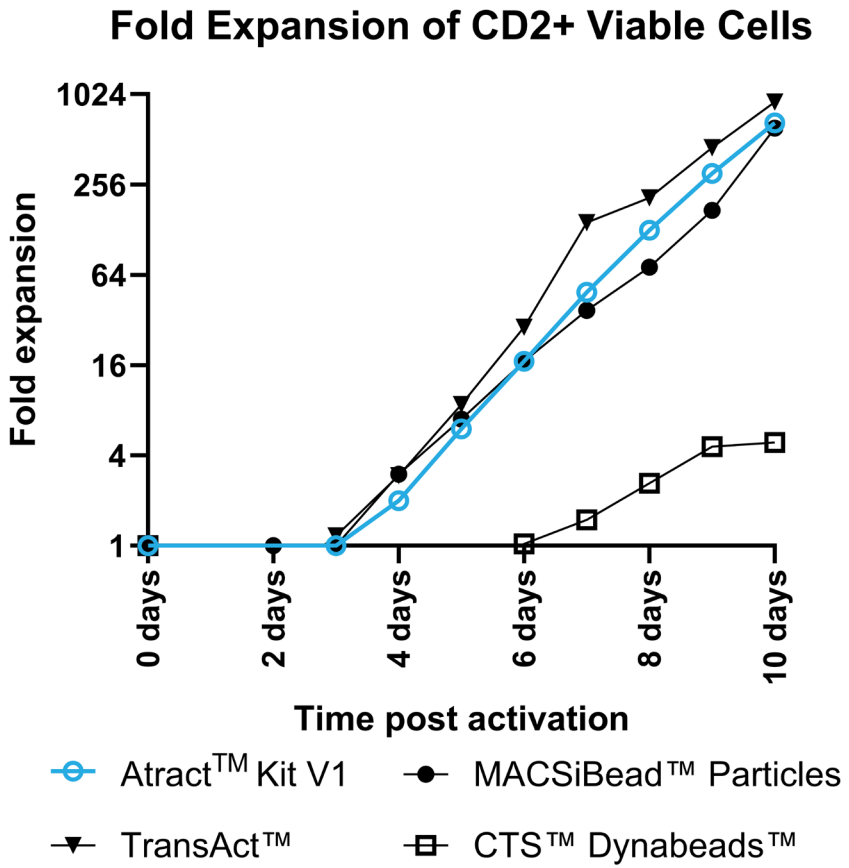


The image shows T-cell expansion after activation with the **Atract™ Kit V1**.

FOLD EXPANSION OF CD2+ CELLS

Fold expansion of CD2+ cells quantifies the increase in cell numbers resulting from successive rounds of cell division. It expresses the final cell count as a multiple or "fold" of the initial count, providing a measure of cell population growth or proliferation.

Result:
In terms of fold expansion, **Atract™ Kit V1** showed comparable performance to MACSiBead™ Particles and TransAct™, whereas CTS™ Dynabeads™ exhibited a significantly lower expansion rate.

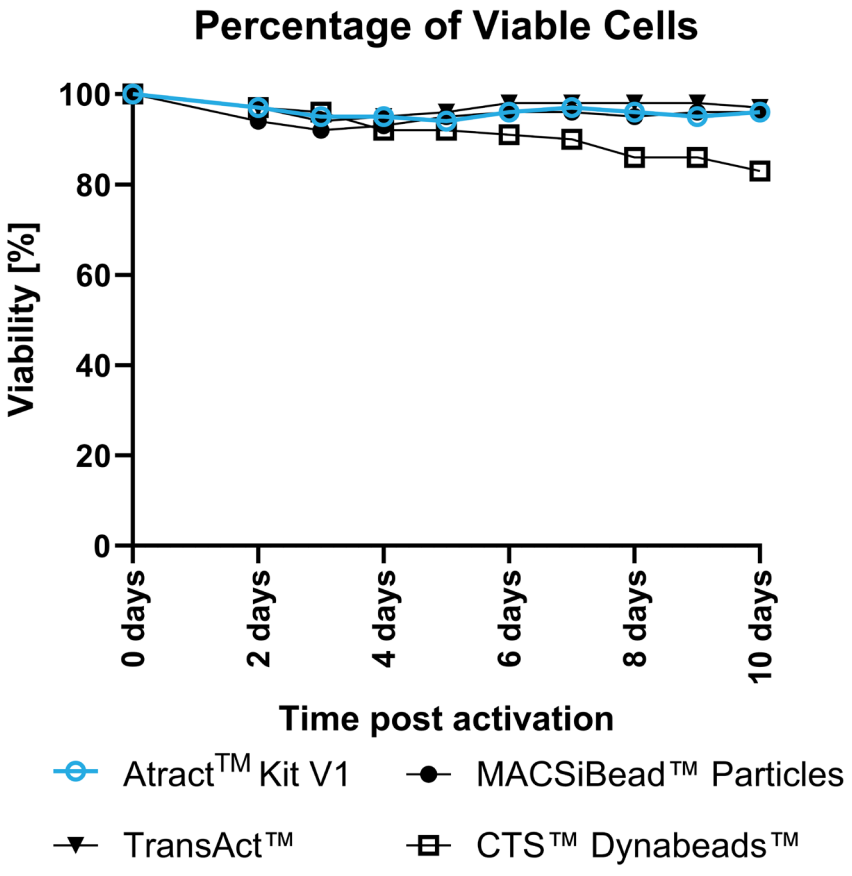


Fold CD2+ Expansion: Fold expansion of CD2+ cells measured before and 2, 3, 4, 5, 6, 7, 8, 9, and 10 days post activation. The average of three biological repeats is shown.

CELL VIABILITY

Cell viability is assessed to ensure that components of the kit are not cytotoxic and do not reduce the overall yield.

Result:
The viability of cells remained largely unaffected across all conditions tested..



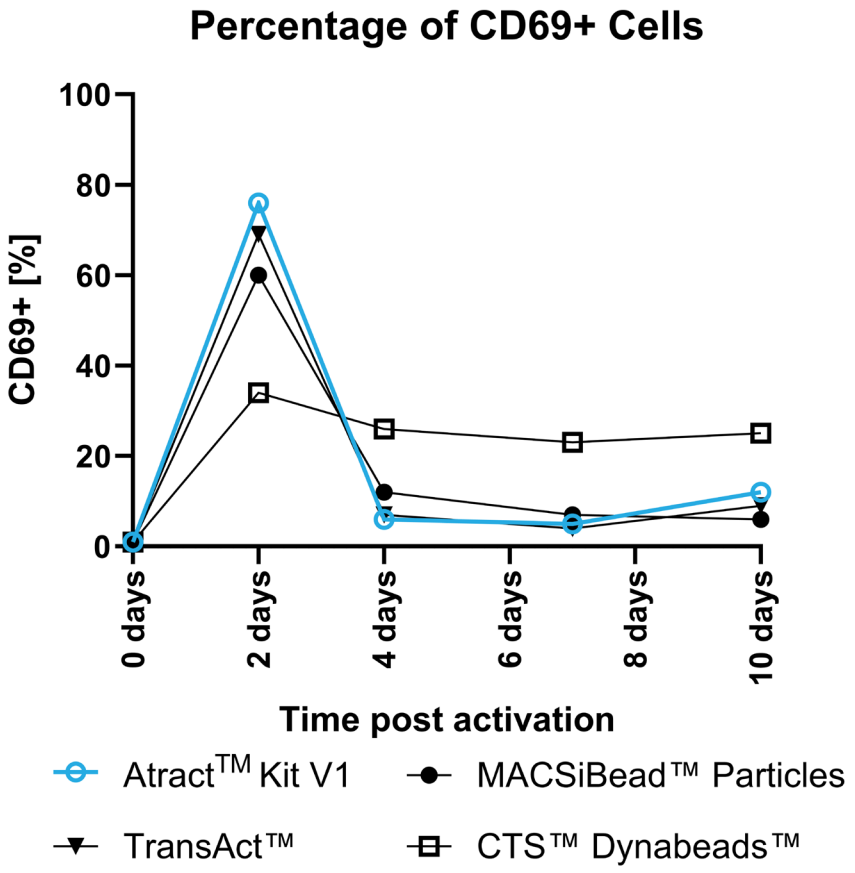
Cell Viability: Cell viability measured before and 2, 3, 4, 5, 6, 7, 8, 9, and 10 days post activation. The average of three biological repeats is shown.

CD69 POSITIVE CELLS

CD69 is an early activation marker found on various immune cells that is rapidly upregulated within a few hours after activation.

Result:
Percentage of CD69+ cells is comparable between **Atract™ Kit V1**, TransAct™, and MACSiBead™ Particles. Activation with our **Atract™ Kit V1** results in the highest percentage of CD69 positive cells on day 2, followed by TransAct™, and then MACSiBead™ Particles. The percentage of CD69+ cells then decreases on days 7 and 10. Activation with CTS™ Dynabeads™ results in a lower but constant percentage of CD69+ cells from day 2 to day 10 post activation.

CD69+ Cells: The percentage of CD69+ cells measured before and 2, 4, 7, and 10 days post activation. The average of three biological repeats is shown.

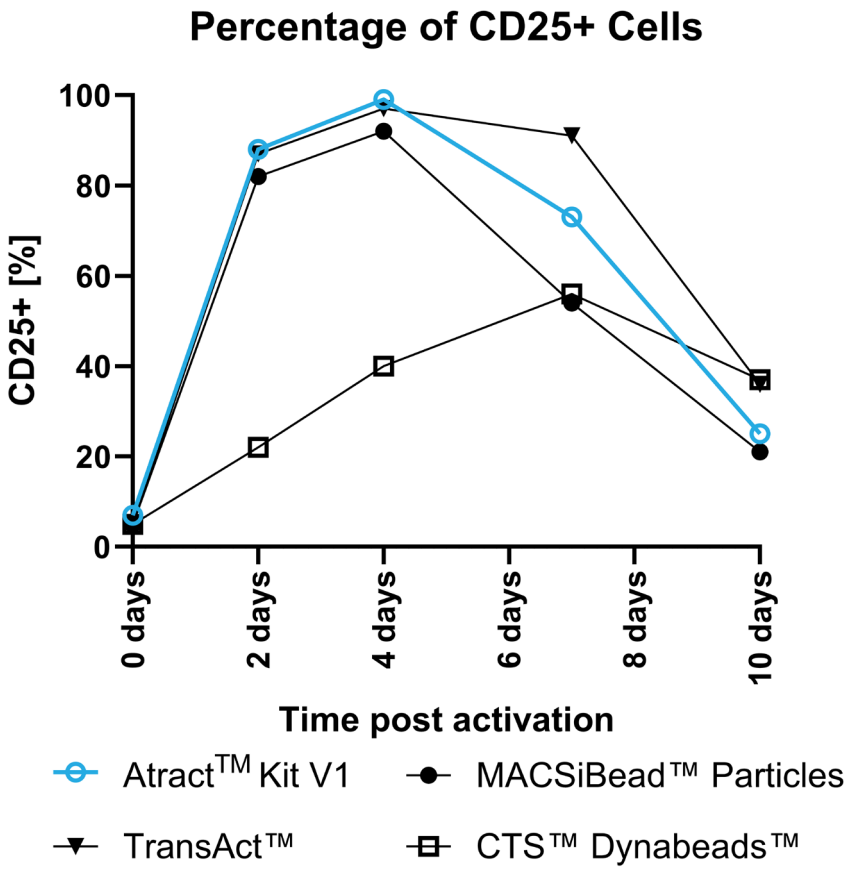


CD25 POSITIVE CELLS

The upregulation of CD25 on T-cells following activation can occur relatively quickly, sometimes as early as a few hours after T-cell activation. However, the level of CD25 expression continues to increase over the course of 24 hours or more, especially if the activation signal is sustained. This upregulation of CD25 is important for the ability of T-cells to respond to cytokines like IL-2, which promote their proliferation and differentiation.

Result:
Expression of CD25 is generally comparable between **Atract™ Kit V1**, TransAct™ and MACSiBeads™, while Dynabeads™ show a lower CD25+ percentage. Population of cells expressing CD25 after incubation with **Atract™ Kit V1** represented around 90% of all cells on day 2 and nearly 100% on day 4, revealing a near ubiquitous activation of T-cells.

CD25+ Cells: The percentage of CD25+ cells measured before and 2, 4, 7, and 10 days post activation. The average of three biological repeats is shown.

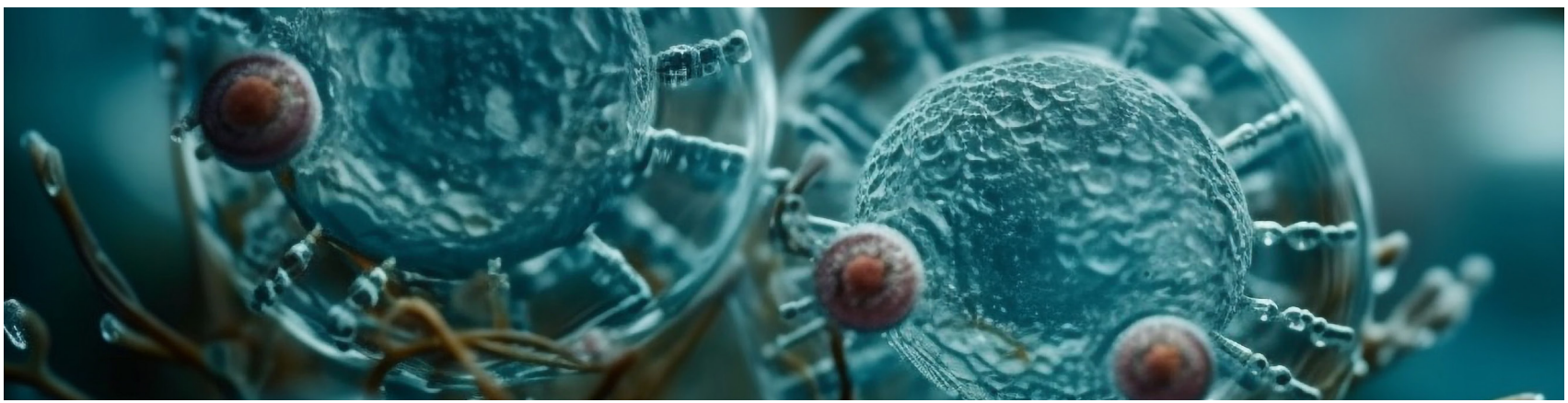


EXHAUSTION MARKERS

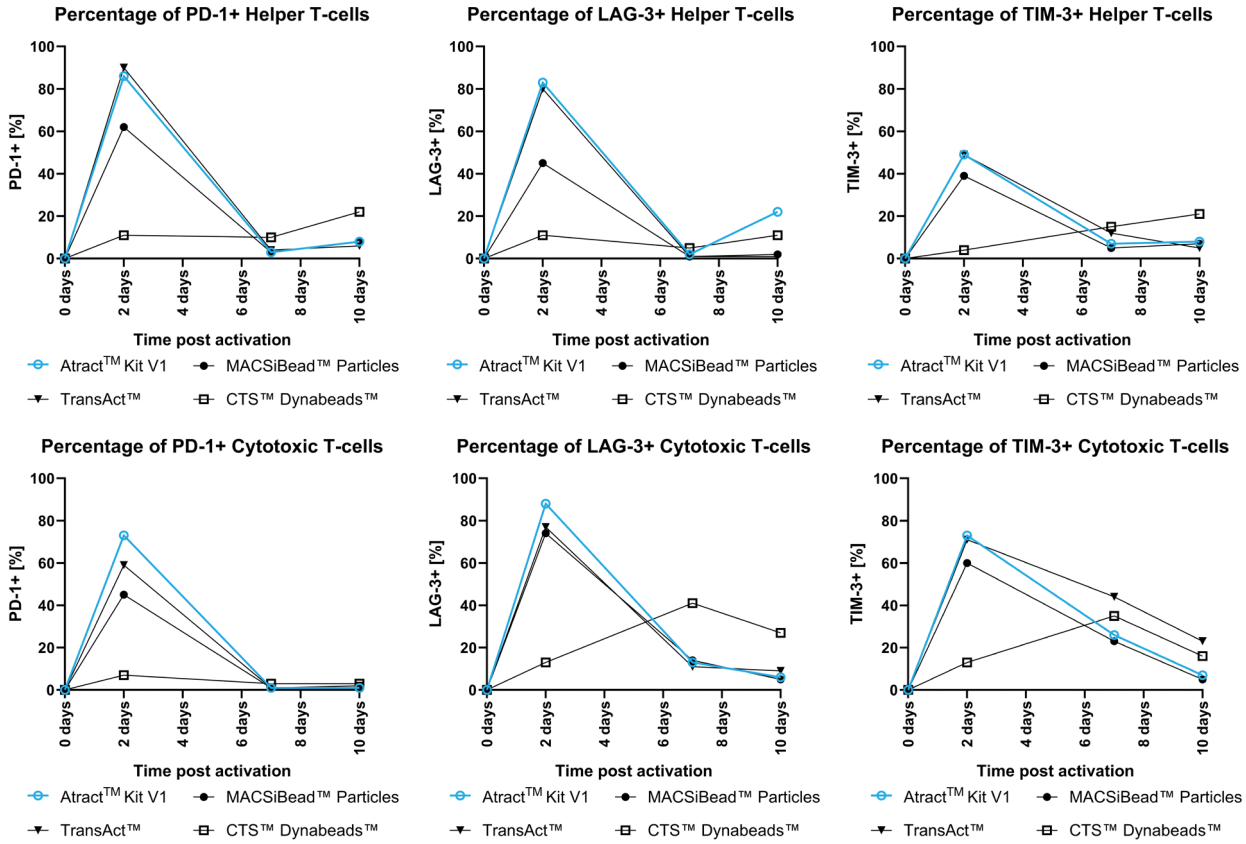
Programmed cell death protein 1 (PD-1), T-cell immunoglobulin and mucin domain-containing protein 3 (TIM-3), and lymphocyte activation gene 3 (LAG-3) are immune checkpoint receptors that play a crucial role in regulating T-cell responses.

During normal immune response, T-cells expand, target, and eliminate infected or abnormal cells. As part of the normal regulatory mechanism, immune checkpoint receptors like PD-1, TIM-3, and LAG-3 are upregulated to prevent excessive immune responses and maintain immune homeostasis. However, prolonged and sustained expression of these checkpoint receptors can lead to T-cell exhaustion. T-cell exhaustion is a state of dysfunction where T-cells lose their effector functions and become less responsive to antigen stimulation. Therefore, while transient upregulation of PD-1, TIM-3, and LAG-3 may indicate a normal immune response to activation, prolonged expression is often associated with T-cell exhaustion and impaired immune function.

Result:
Proportion of cells expressing all exhaustion markers (PD-1, LAG-3 and TIM-3) followed a similar trend after activation with **Atract™ Kit V1,** TransAct™ and MACSiBeads™, with a spike in expression on day 2 and a subsequent decrease. Activation with Dynabeads™ showed a different trend of exhaustion marker expression, indicating a delayed response in comparison to other tested kits.



EXHAUSTION MARKERS



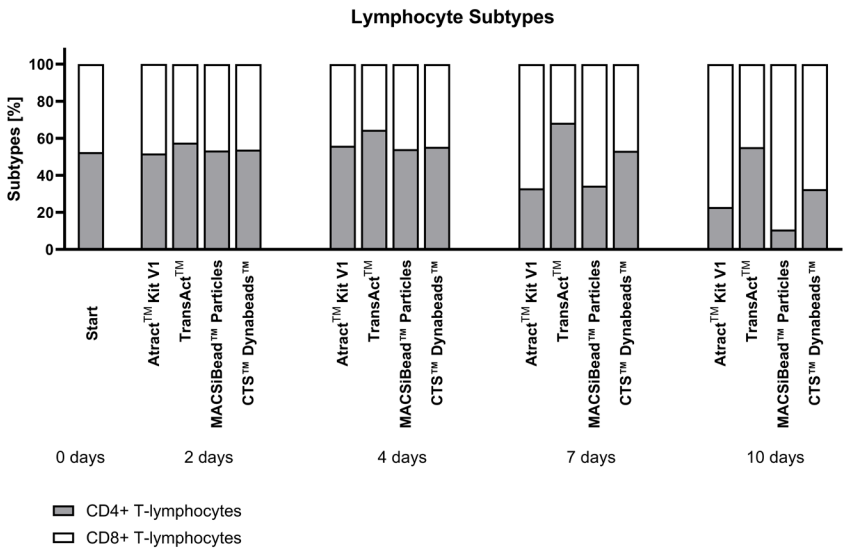
Exhaustion markers: PD-1, LAG-3, and TIM-3 positive cells during 10 days of expansion. Top: helper T-cells; bottom: cytotoxic T-cells.

LYMPHOCYTE SUBTYPES DURING ACTIVATION

Lymphocytes consist of several subtypes, performing different important functions during activation and expansion, with diverse responses to activating stimuli. CD19 is a marker for B-cells while CD4 and CD8 denote helper and cytotoxic T-cells, respectively.

Result:
A lower CD4:CD8 ratio was observed with **Atract™ Kit V1** and MACSiBead™ Particles compared to TransAct™ and CTS™ Dynabeads™. The percentage of B-cells decreased in all samples.

The CD19+ population (data not shown) was measured at 10% at the start. By day 10 of expansion, this populations had reduced to near-zero levels. **Atract™ Kit V1,** MACSiBead™ Particles and CTS™ Dynabeads™ had shown comparable CD4:CD8 ratios by day 10 post activation, while TransAct™ had a higher proportion of CD4 positive cells.



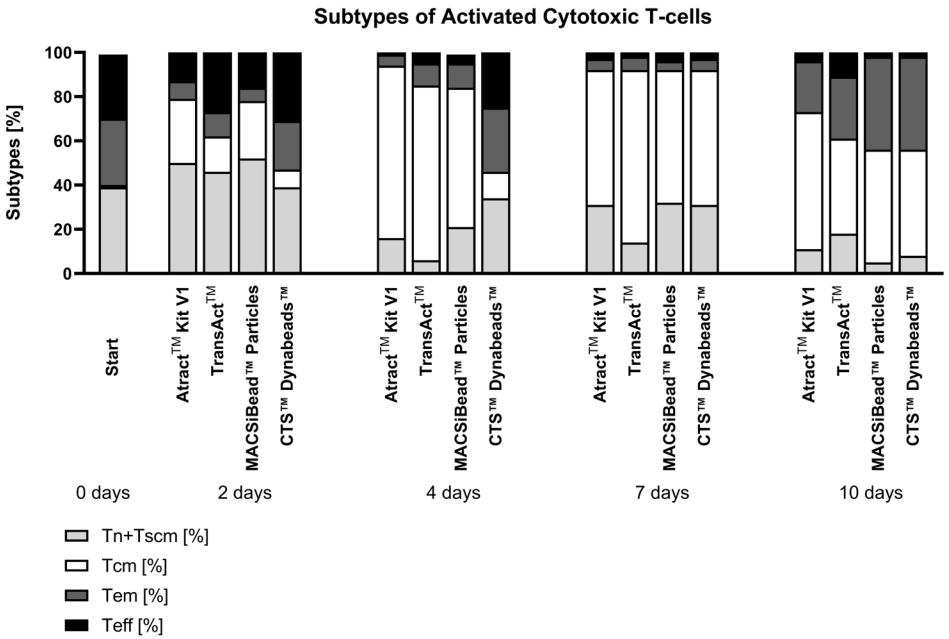
Lymphocyte Subtypes: CD4 and CD8 lineage markers were measured before and 2, 4, 7, and 10 days post activation. The average of three biological repeats is shown.

DIFFERENT FUNCTIONAL SUBSETS OF T-CELLS

Different T-cell populations have unique characteristics and functions during an immune response, making them more or less suitable for various cell therapy applications.

Result:

A significant increase in the percentage of central memory T-cells was observed by day 4 of activation with **Atract™ Kit V1** compared to other tested kits. This coincided with a reduction in other populations. By day 10, the proportion of effector memory cells had increased in all samples. **Atract™ Kit V1** resulted in the highest percentage of central memory cells, while TransAct™ led to more effector and naive cells.



T-cell Differentiation: Differentiation of the CD8+ T-cells is shown. Naive and T memory stem cells (Tn + Tscm), central memory (Tcm), effector memory (Tem), and effector T-cells (Teff) were analyzed by multicolor flow cytometry.

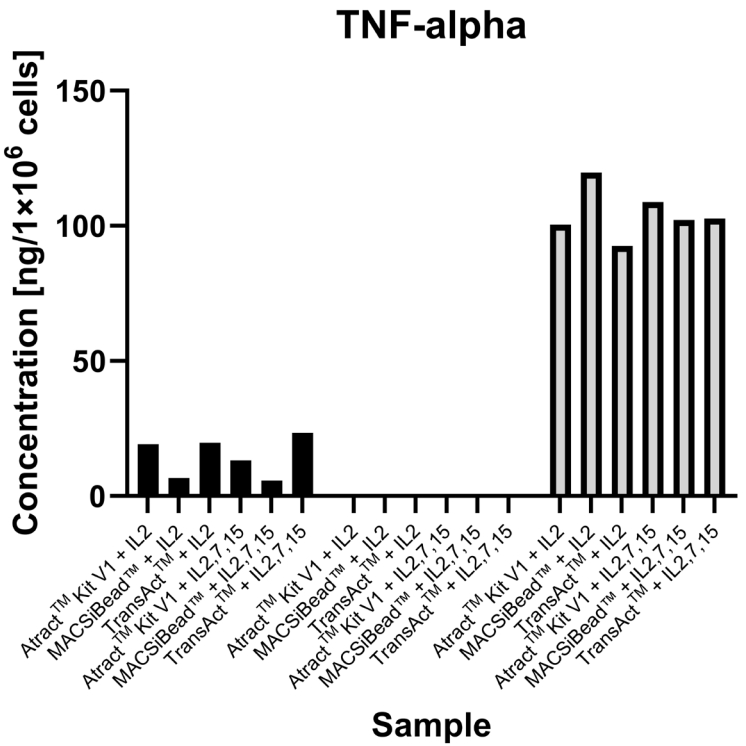
PRELIMINAR FUNCTIONAL TEST: TNF-ALPHA AND IFN-GAMMA

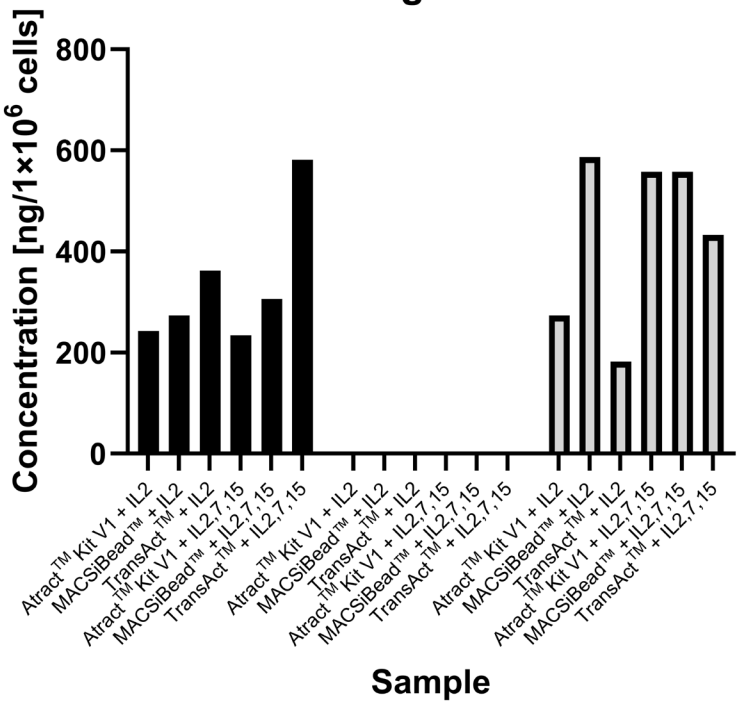
Tumor necrosis factor alpha (TNF-alpha) and interferon gamma (IFN-gamma) are cytokines produced by various cells, including T-cells. TNF-alpha is a proinflammatory cytokine that contributes to the activation of other immune cells and the initiation of inflammatory processes. IFN-gamma has both antiviral and immunomodulatory functions. It is involved in enhancing the cytotoxicity of T-cells, promoting antigen presentation, and activating macrophages. TNF-alpha and IFN-gamma are key cytokines produced by activated T-cells, especially during the early phases of immune response.

Monitoring the production of these cytokines is a valuable approach to assess functionality and responsiveness of T-cells *in vitro*.

Result:
Cells activated with all tested kits secreted TNF-alpha and IFN-gamma into the culture medium. On day 10 post activation, the TNF-alpha and IFN-gamma levels were under the detection limit; however, upon reactivation, T-cells resumed production of both cytokines. Reactivation resulted in similar levels of IFN-gamma and TNF-alfa between all tested kits.

We have demonstrated that T-cells retain the ability to produce TNF-alpha and IFN-gamma after expansion. This confirms that T-cells activated with **Atract™ Kit V1** maintain their primary function.





- 3 days post activation
- 10 days post activation
- 1 day post reactivation (day 10)

- 3 days post activation
- 10 days post activation
- 1 day post reactivation (day 10)

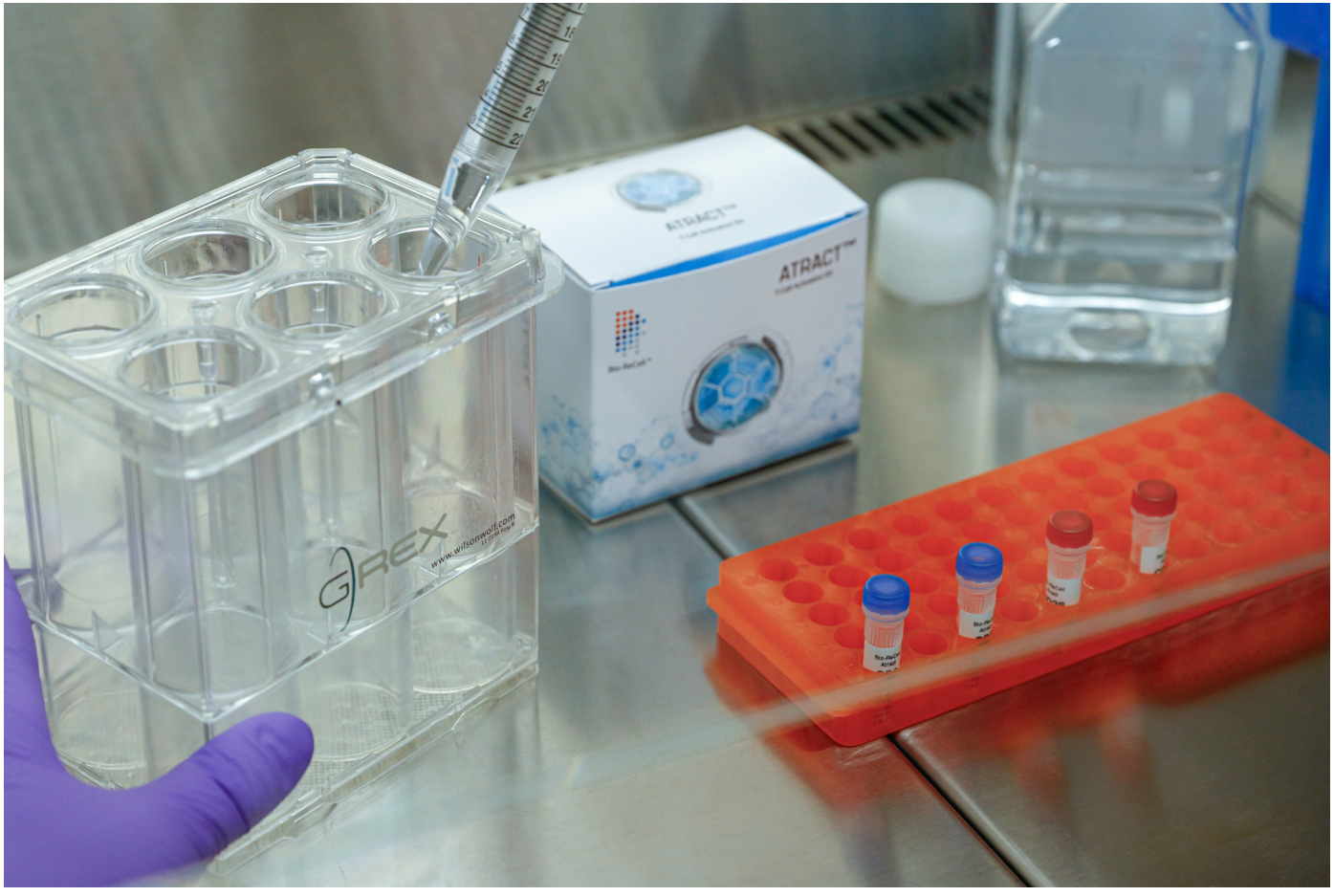
RESULTS BREAKDOWN

Experimental Design: G-Rex Platform

To evaluate the performance of **Atract™ Kit** products in a large-scale setting, we transitioned to the G-Rex platform, a standard in T-cell production known for its simplicity, scalability, and cost-effectiveness. The ability of **Atract™ Kit V1** and **V2** to expand T-cells was then compared to the widely used Miltenyi T Cell TransAct™ activation kit.

Method:

Step	Time	Description
Activation Medium	Day 0	CTS AIM-V medium was mixed with 10% FBS, PenStrep with antimycotics, and 200 U/mL IL-2.
Cell Preparation	Day 0	PBMCs from 3 healthy donors were isolated and diluted to 10 M cells/mL.
Atract Preparation	Day 0	The selected quantities of **Atract™** CD3 and **Atract™** CD28 reagent were mixed, washed in 1 mL of medium, and then put into 1 mL of medium. For TransAct, 50 µL of TransAct reagent was mixed with 0.95 mL of medium.
Activation Setup	Day 0	1 mL of cells, 1 mL of activation reagent, and 3 mL of medium were mixed and pipetted into each well of a 6-well G-Rex plate. The plates were then transferred to the incubator (37 °C, 5% CO_2).
Expansion	Day 2	95 mL of medium was slowly added to each well. Culture plates were transferred to the incubator (37°C, 5% CO_2).
Flow Cytometry Analysis	Day 9	80 mL of medium was removed. Plates were swirled gently for 30 seconds. Small samples (200 µL) were analyzed by multicolor flow cytometry.

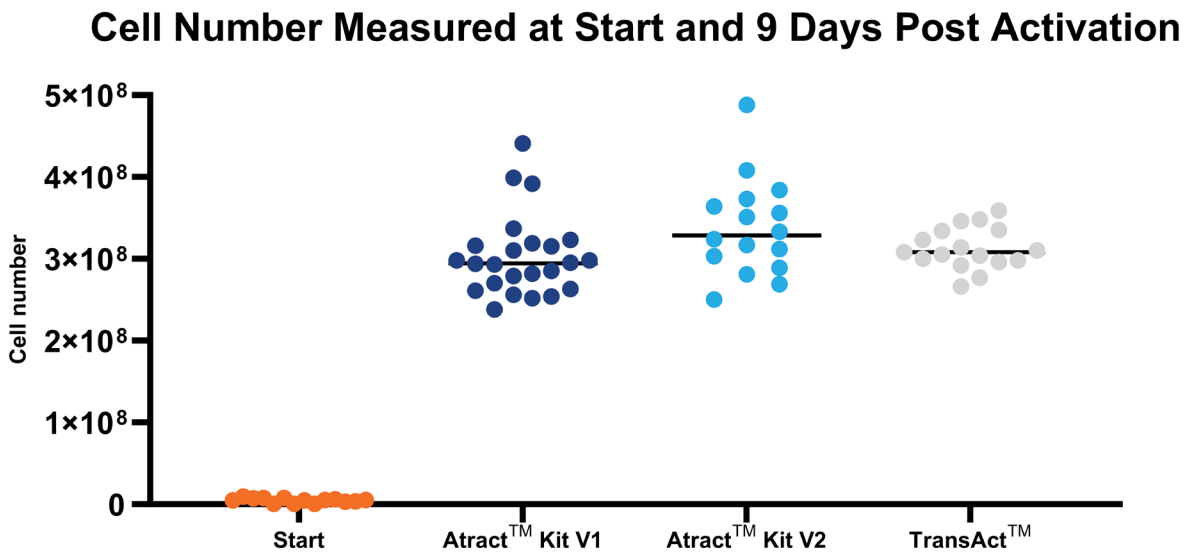


G-Rex6M Well Plate

CELL NUMBER

The number of CD2+ cells represents the final cell count as an average of 3 measurements analyzed by flow cytometry, providing an assessment of cell population growth or proliferation.

Result:
Atract™ Kit V1 demonstrated similar results to TransAct™, while **Atract™ Kit V2** had a slightly higher cell number.

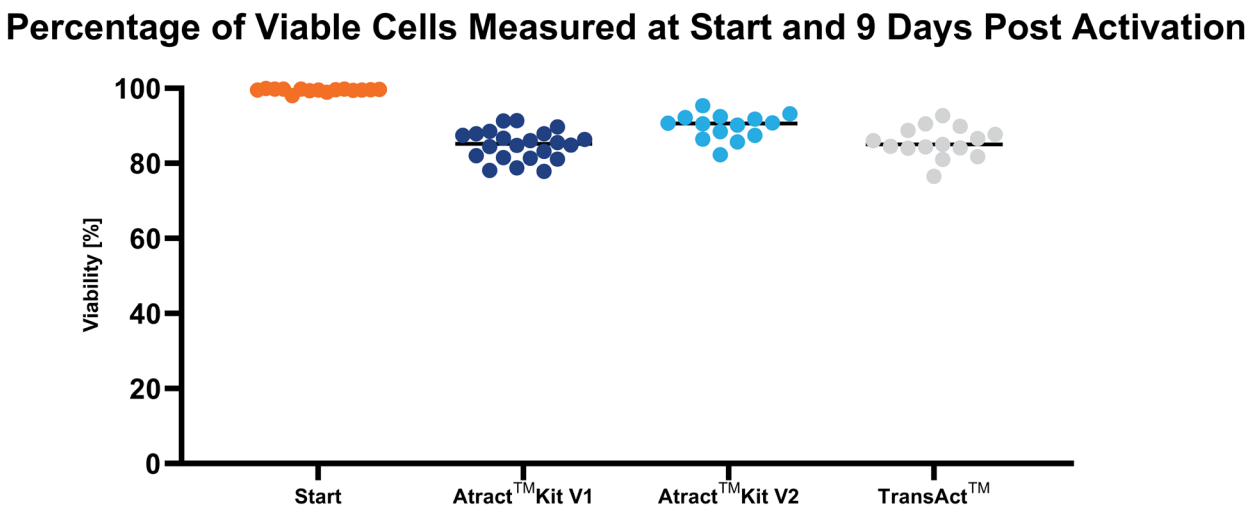


Cell number: The number of CD2+ cells measured at start and 9 days post activation.

CELL VIABILITY

Cell viability is assessed to ensure that components of the kit are not cytotoxic and do not reduce overall cellular yield.

Result:
The viability of cells was not significantly affected under any condition, although cells expanded with **Atract™ Kit V2** displayed a slightly higher viability..

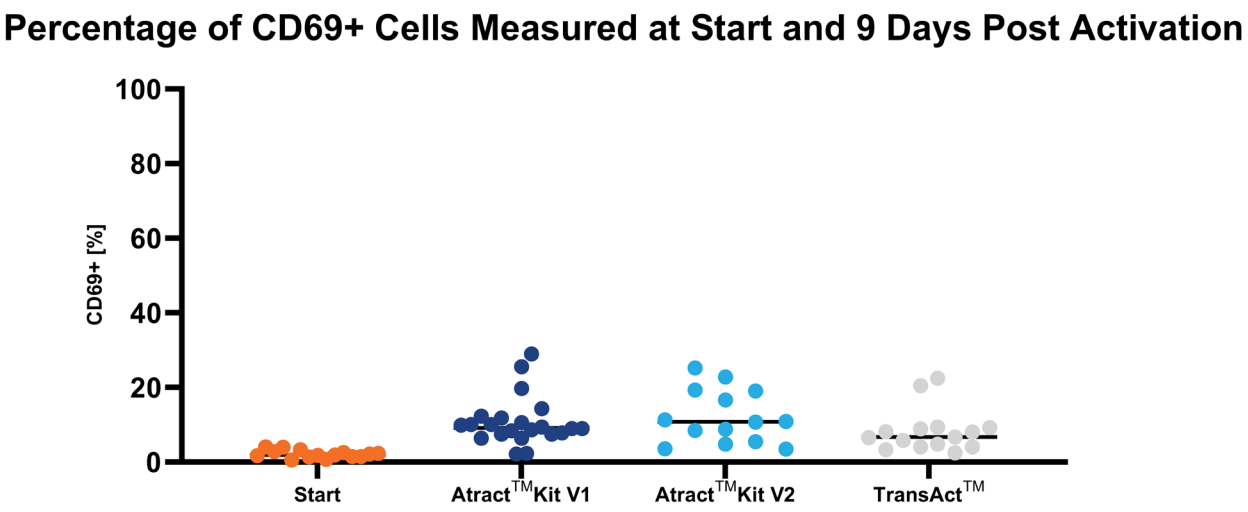


Viability: Cell viability measured at start and 9 days post activation.

CD69 POSITIVE CELLS

CD69 is an early activation marker found on various immune cells, and is rapidly upregulated within a few hours after activation. In contrast to CD25, due to the transcient nature of its expression, CD69 percentage decreases despite continuous activation stimulus.

Result:
The percentage of CD69+ cells after 9 days of expansion was comparable between **Atract™ Kit V1** and TransAct™. **Atract™ Kit V2** displayed a slightly higher CD69+ percentage.

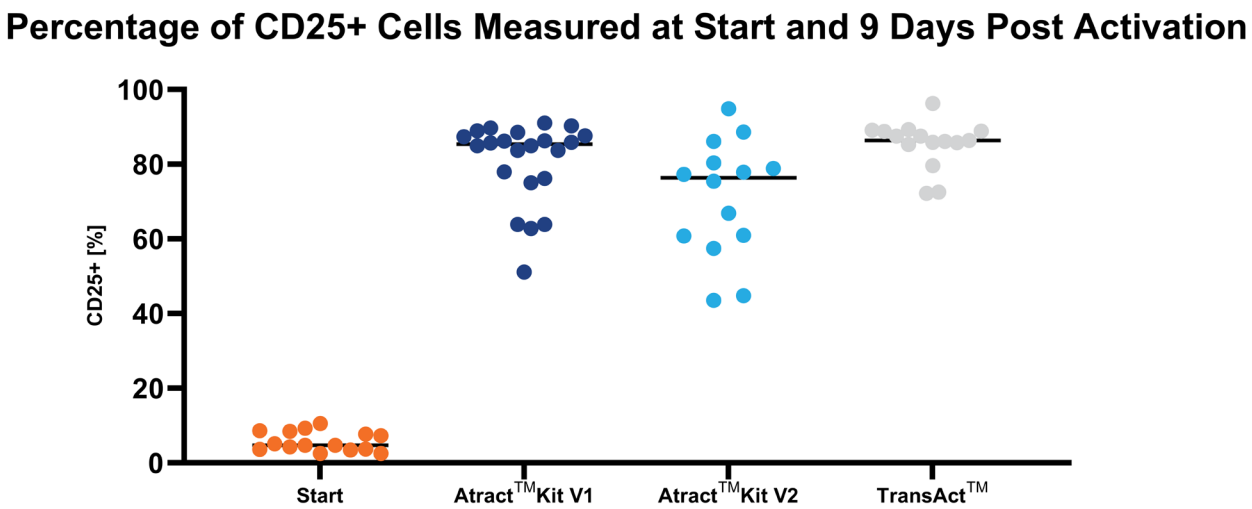


CD69+ Cells: The percentage of CD69+ cells measured at start and 9 days post activation.

CD25 POSITIVE CELLS

The upregulation of CD25 on T-cells following activation can occur relatively quickly, sometimes as early as a few hours after T-cell activation. However, the level of CD25 expression continues to increase over the course of 24 hours or more, especially if the activation signal is sustained.

Result:
The percentage of CD25 expressing cells remained elevated throughout the experiment. Expression levels of CD25 were comparable between **Atract™ Kit V1** and TransAct™, while **Atract™ Kit V2** displayed a slighty lower CD25+ percentage.



CD25+ Cells: The percentage of CD25+ cells measured at start and 9 days post activation.

EXHAUSTION MARKERS

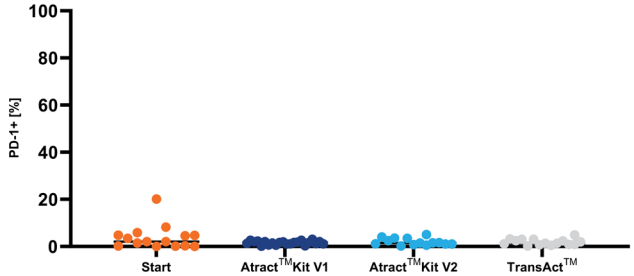
Programmed cell death protein 1 (PD-1), T-cell immunoglobulin and mucin domain-containing protein 3 (TIM-3), and lymphocyte activation gene 3 (LAG-3) are immune checkpoint receptors that play a crucial role in regulating T-cell responses.

During normal immune response, T-cells expand, target, and eliminate infected or abnormal cells. As part of the normal regulatory mechanism, immune checkpoint receptors like PD-1, TIM-3, and LAG-3 are upregulated to prevent excessive immune responses and maintain immune homeostasis. However, prolonged and sustained expression of these checkpoint receptors can lead to T-cell exhaustion. T-cell exhaustion is a state of dysfunction where T-cells lose their effector functions and become less responsive to antigen stimulation. Therefore, while transient upregulation of PD-1, TIM-3, and LAG-3 may indicate a normal immune response to activation, prolonged expression is often associated with T-cell exhaustion and impaired immune function.

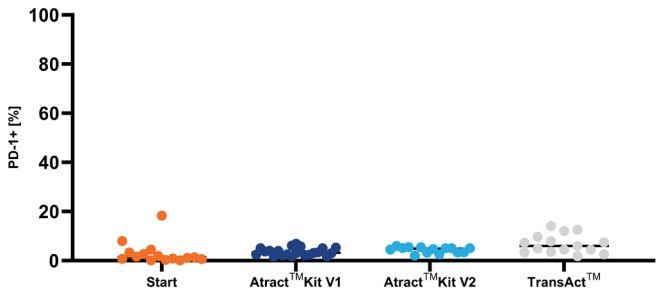
Result:
Expression levels of exhaustion markers (PD-1, LAG-3, and TIM-3) after 9 days of expansion were similar with **Atract™ Kit V1** and **V2** and TransAct™. A slightly lower percentage of CD366+ was observed with **Atract™ Kit V2**.

Exhaustion markers: PD-1, LAG-3, and TIM- 3 positive cells were measured at start and 9 days post activation. Right: helper T-cells; Left: cytotoxic T-cells.

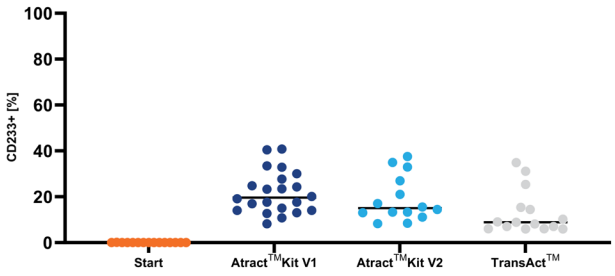
Percentage of PD-1+ of Cytotoxic T-cells Measured at Start and 9 Days Post Activation



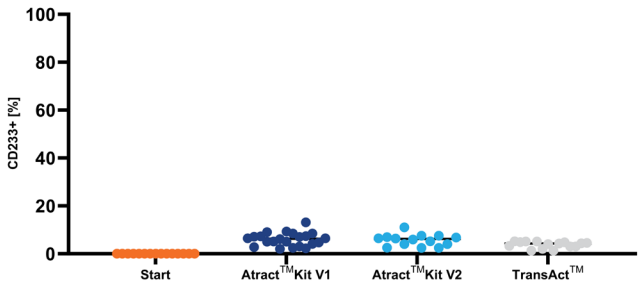
Percentage of PD-1+ of Helper T-cells Measured at Start and 9 Days Post Activation



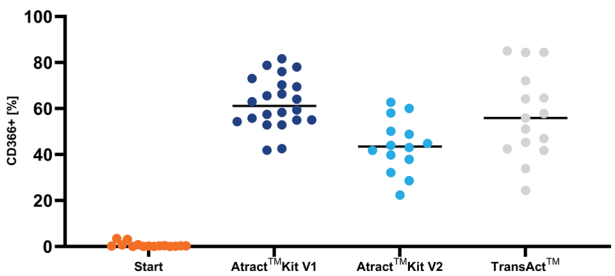
Percentage of CD223+ of Cytotoxic T-cells Measured at Start and 9 Days Post Activation



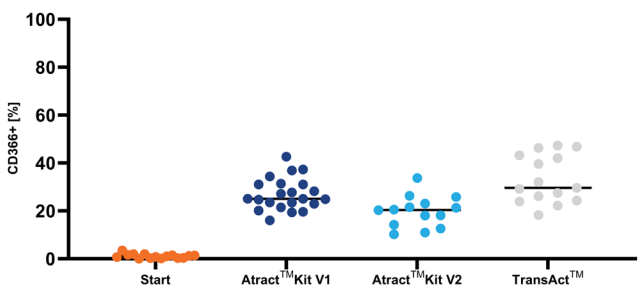
Percentage of CD223+ of Helper T-cells Measured at Start and 9 Days Post Activation



Percentage of CD366+ of Cytotoxic T-cells Measured at Start and 9 Days Post Activation



Percentage of CD366+ of Helper T-cells Measured at Start and 9 Days Post Activation

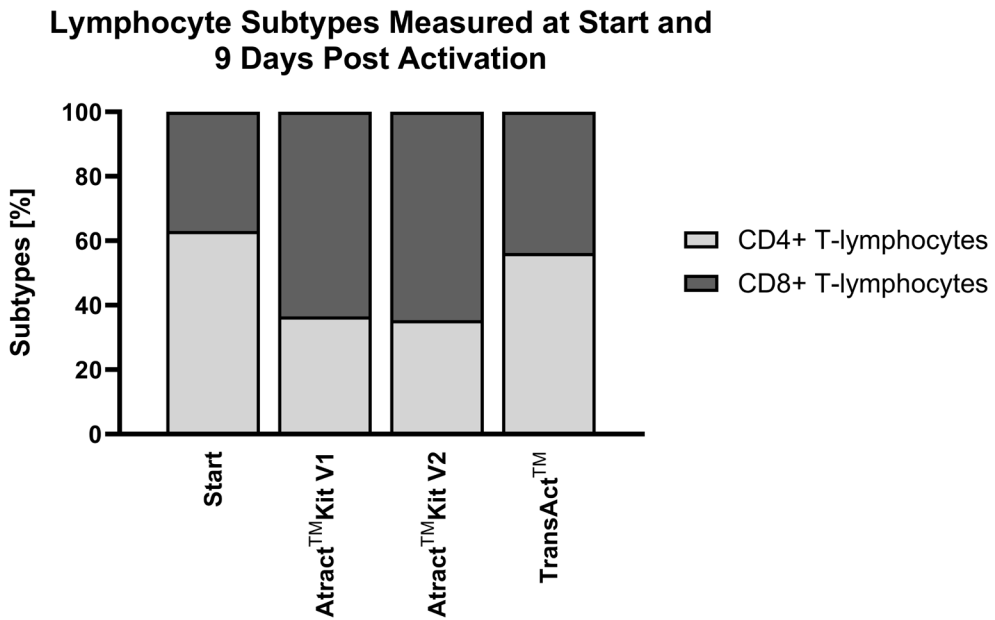


LYMPHOCYTE SUBTYPES

Lymphocytes consist of several subtypes, performing different important functions during activation and expansion, with diverse responses to activating stimuli. CD19 is a marker for B-cells while CD4 and CD8 denote helper and cytotoxic T-cells respectively.

Result:
More cytotoxic (CD8+) T-cells were observed with **Atract™ Kit V1** and **V2** in comparison to TransAct™.

At start, the CD19+ population (data not shown) was measured at 5%. By day 9, the population decreased with all methods, falling to below 0.2%.



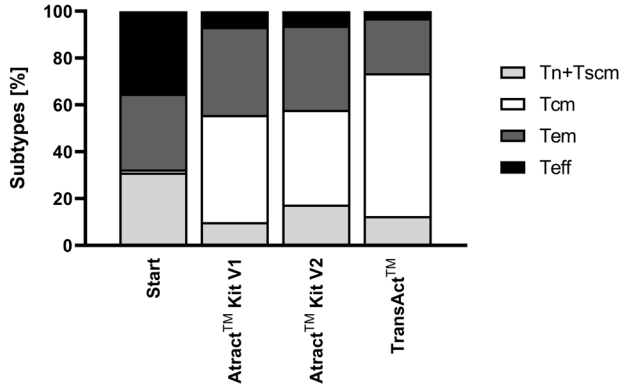
Lymphocyte Subtypes: CD4 and CD8 lineage markers were measured at start and 9 days post activation.

DIFFERENT FUNCTIONAL SUBSETS OF T-CELLS

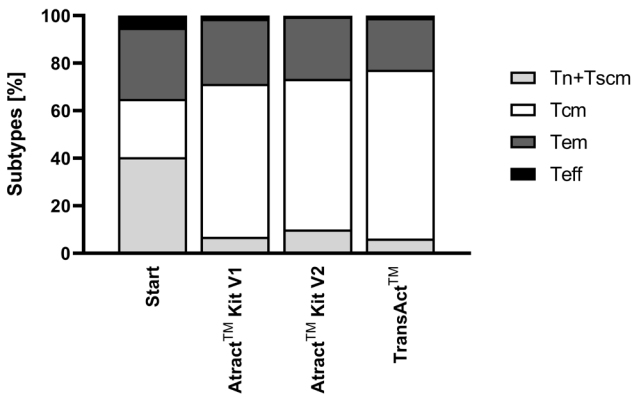
Different T-cell populations have unique characteristics and functions during an immune response, making them more or less suitable for various cell therapy applications.

Result:
Activation with **Atract™ Kit V1** and **V2** led to higher percentages of effector memory T-cells in comparison to TransAct™ on day 9. **Atract™ Kit V2** resulted in more naive and T memory stem cells, while TransAct™ resulted in more central memory T-cells.

Functional Subtypes of Cytotoxic T-cells Measured at Start and 9 Days Post Activation



Functional Subtypes of Helper T-cells Measured at Start and 9 Days Post Activation



T-cell Differentiation: Differentiation of cytotoxic and helper T-cells is shown. Naive and T memory stem cells (Tn + Tscm), central memory (Tcm), effector memory (Tem), and effector T-cells (Teff) were measured at start and 9 days post activation. Top: cytotoxic T-cells; Bottom: helper T-cells.

CONCLUSIONS

Drawing on the results of our assays, we conclude that **Atract™ Kit** products are potent activators of T-cells, as evidenced by increased CD69 and CD25 expression. Furthermore, **Atract™ Kit V1** was equal to, and even slightly outperformed, competitors on metrics such as fold expansion of T-cells and activation marker expression. **Atract™ Kit** products offer the benefits of fine-tunable cell activation, hassle-free removal of the reagents and leaving behind a pure cell sample with no residuals. **Atract™ Kit** is an indispensable tool for T-cell activation research.

Atract™ Kits offer several advantages over our competitors, summarised here:

	Atract™ Kit V1	**Atract™ Kit V2**	MACSiBeads™	TransAct™	Dynabeads™
Covalently Bound Antibodies	Yes	Yes	No	No	No
No Magnets, No Residual Antibodies, Just Pure Cells	Yes	Yes	No	No	No
Carriers Cannot Be Phagocytized	Yes	Yes	No	No	No
Option to Fine-Tune CD3 to CD28 Bead Ratio	Yes	No	Yes	No	No
Special Equipment Required	No	No	Tube rotator, MACSiMAG™ Separator	No	CTS™ DynaMag™ Magnet, Mixer
Activation Reagent Preparation Steps	Combining, Washing	Washing	Loading, Washing	No	Washing
Preparation Time	50 Minutes	40 Minutes	180 Minutes	30 Minutes	40 Minutes

TO ORDER YOUR **ATRACT™ KIT**
OR TO SPEAK TO OUR EXPERTS CONTACT US

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