WHITE PAPER

Bio-ReCell®

No Compromise in Cell Expansion: Atract™ Kit T-Cell Activation Reagent Performance in Closed G-Rex® Systems

INTRODUCTION

Bio-ReCell, in collaboration with ScaleReady, conducted a study to evaluate the Atract™ T-Cell Activation Kit when transitioning from an open system (OS) workflow to a closed system (CS) workflow. Closed system cell processing is a crucial part of cell therapy manufacturing, as high safety standards, stringent QC and deviation avoidance require highly standardised and thought-out manufacturing solutions. Closed system protocols and products are designed to ensure high sterility, minimised contamination risk as well as operator safety. They are key for GMP settings, where sterility is a prerequisite for product quality and patient safety.

This study evaluates the performance of the Atract™ Kit in a closed-system G-Rex® configuration, comparing it to an open system setup. Specifically, it assesses whether differences in handling, mixing conditions, and reduced manual intervention associated with closed processing impact key performance parameters of the Atract™ Kit. A prototype of Closed System Atract™ Kit format was used in the study. The reagent is a biocompatible particle suspension, packaged in a welded, clamped tube. The product is designed to provide a seamless activation process with a minimised number of required steps and reagent handling.

OBJECTIVE

The objective of this study was to assess whether transitioning from an open to a closed G-Rex® system affects the performance of the Atract™ Kit, as measured by cell expansion, viability, and phenotype.

METHODS

CELL SOURCE & PREPARATION

PBMCs from three healthy donors were isolated using Histopaque-1077 density gradient centrifugation. Red blood cells were lysed using BD Pharm Lyse™ Lysing Buffer, and viable mononuclear cells were quantified using flow cytometry.

MEDIUM & REAGENTS

GMP Human T Cell Activation Medium (CCM038-GMP, Bio-Techne) was supplemented with 5 % Plasma-derived Human AB Serum, antibiotics, and 200 IU/mL Animal-Free rhIL-2 (BT-002-AFL, Bio-Techne). Atract™ T-Cell Activation Kit V2 was used as activation reagent.

ACTIVATION SETUP

Open system: Cells and Atract™ Kit particles were combined and seeded into G-Rex®100M Open system vessels according to the conditions outlined in Table 1. On day 0, cells were seeded at a concentration of 1 × 10^6 cells/mL in 5% of the total G-Rex®100M volume (0.5 × 10^6 cells/cm²). 160 mg of Atract™ Kit was added directly to the cell suspension, mixed, and transferred into the G-Rex®100M using a 50 mL serological pipette.

Cultures were incubated at 37 °C with 5 % CO_2. On day 2, the remaining 95 % of the culture medium was added to reach the final working volume of the G-Rex®100M, followed by a further 7-day expansion, for a total incubation period of 9 days.

Closed system: Cells were prepared in activation medium within a sterile bag equipped with PVC tubing. This was aseptically connected via tube welding to a pre-production prototype of the closed-system format of Atract™ Kit, which was subsequently connected to a closed-system G-Rex®100M. The G-Rex®100M-CS was gravity-filled with the cell suspension, effectively flushing the activation particles into the G-Rex®. This process design requires minimal operator manipulation, with the whole process being done in a fully closed manner (Figure 1). After transfer, the tubing was sealed, and the G-Rex®100M-CS was incubated at 37 °C with 5 % CO_2.

On day 2, the remaining 95 % of the culture medium was added to reach the final working volume using a closed transfer approach. Cultures were then maintained under standard conditions for an additional 7-day expansion period, for a total incubation period of 9 days.

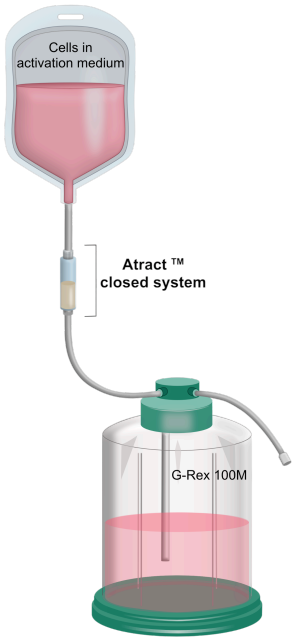


Figure 1: Closed system activation setup with Atract™ Kit.

CONDITIONS	Atract™ Kit reagent (mg)	Cells/flask (x 10^6)	Starting media volume day 0 (mL)	Media volume addition on day 2 (mL)
G-Rex®100M Closed system or Open system	160	50	50	950

Table 1: Conditions

HARVESTING & ANALYSIS (day 9 or day 7 of expansion)

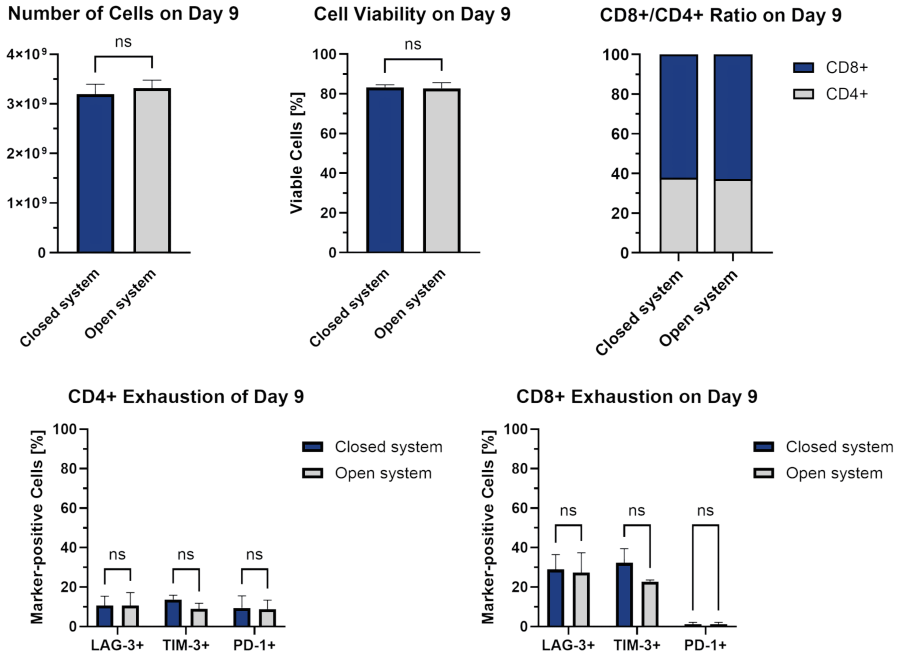
Open system: Approximately 90 % of the culture medium was removed and the remaining cell suspension was collected and filtered through a 30 µm filter to remove Atract™ Kit particles.

Closed system: Medium removal and cell collection was done by the GatheRex Cell Harvest Pump (P/N: 80000E). First, 90 % of the spent activation medium was removed via the G-Rex® closed-system media reduction line into a collection bag. The remaining cell suspension was resuspended by gentle agitation of the G-Rex®100M-CS and subsequently transferred via the cell harvest line through a 30 µm filter into a separate collection bag.

Aliquots from all biological repeats, CS and OS, were analyzed with flow cytometry. Key markers for activation (CD25, CD69), subset distribution (CD4, CD8, CD19, CD45RA, CCR7), and exhaustion (PD-1, TIM-3, LAG-3) were assessed.

RESULTS

Final cell counts after a 9-day expansion period were comparable between the closed system and open system. Both culturing protocols achieved >3*10^9 cells with non-significant differences. Cell viability was consistently maintained above 80 % in both systems. The CD4/CD8 ratio showed similar values between the CS and OS across all three biological replicates. Exhaustion markers (PD-1, TIM-3, and LAG-3) were present at low levels in the closed system, indicating healthy T cell profiles. $CD19^+$ percentage stayed below 0,5 % in both conditions, while functional subtype distributions were similar between the CS and OS platforms (data not shown).



DISCUSSION

The results demonstrate that the Atract™ T-Cell Activation Kit performs consistently across both closed and open system conditions, supporting effective T-cell activation and expansion without negatively impacting phenotype or increasing exhaustion marker expression. The comparable outcomes observed between conditions indicate that the activation reagent functions robustly independent of the manufacturing setup. This suggests that its mechanism of action is stable and reproducible, making it suitable for use in different process configurations.

CONCLUSION

Overall, the Atract™ T-Cell Activation Kit shows reliable and equivalent performance in both closed and open systems, confirming its suitability for flexible integration into T-cell manufacturing workflows.

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