## RAPID HUMAN T CELL EXPANSION USING GAS-PERMEABLE BAGS IN THE EPPENDORF NEW BRUNSWICK™ S41i CO₂ INCUBATOR SHAKER

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Recent advances in cellular therapy have greatly increased the demand for T cell expansion techniques. T cells must be expanded rapidly to achieve high cell densities while maintaining high viability and T cell identity. One of the best cell culture tools for large-scale expansion is stirred-tank bioreactors. However, cell culture in bioreactors requires a relatively large number of cells for inoculation. We developed a -method using gaspermeable bags to produce a sufficient number of T cells for the inoculation of bench-scale bioreactors.

We cultivated T cells in suspension in gas-permeable bags in an Eppendorf New Brunswick S41i CO<sub>2</sub> incubator with built-in shaker. We tested bags from different manufacturers with maximum fill volumes of 120 mL to 5 L.

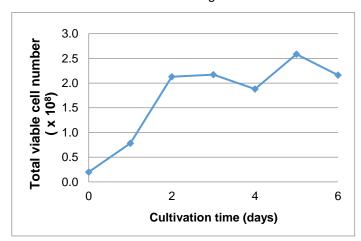


Figure 1 – Expansion of T cells in gas-permeable bag

Furthermore we tested different shaking speeds to identify the optimal condition for T cell expansion. Proper agitation was required as the T cells placed on a stationary platform did not grow. We also explored a more conventional suspension culture method using agitated shake flasks. The method using the bags was much more successful, achieving greater than 10-fold expansion in less than 4 days, compared to 2-3 fold expansion in the shake flasks. In summary, using gas-permeable bags and a CO<sub>2</sub> incubator shaker, we established a method for inoculum preparation for large-scale T cell culture in stirred-tank bioreactors.