T Cell Culture in VueLife® “C” Series Bags

VueLife® “C” Series Bags are Saint-Gobain’s standard series FEP bags for use in various in vitro bioprocessing applications, including expansion of human primary T cells for Cell Therapy.

The aim of this study was to investigate the performance of expanding activated human T cells in VueLife® “C” Series Bags at different fill heights and volumes, and to illustrate the processing steps and available options for using VueLife® bags for expansion cultures.

VUELIFE® “C” SERIES BAGS
VueLife® “C” Series Bags are manufactured using the highest quality USP Class VI fluorinated ethylene propylene (FEP) and are highly permeable to oxygen and carbon dioxide, while being impermeable to water. Each VueLife® “C” Series Bag typically features a needle-less injection site and a “Y” connector with PVC tubing leading to a female luer and a heat-sealed sterile docking tube. This reduces the risk of culture contamination while allowing easy access for the introduction of new material. Other types of ports, tubing, and connections are available and can be customized to the needs of the user.

MATERIALS AND METHODS
For this study, human CD4+/CD8+ T cells were activated and expanded in VueLife® 197-C Series Bags. The main objective of this study was to investigate and compare the performance of human T cell expansion in VueLife® “C” Series Bags at fill volumes at < 1 cm, 1 cm and > 1 cm height.

To compare the impact of the different fill heights on cell performance, T cells were seeded into 197-C bags at different starting volumes of 25 ml, 50 ml, and 75 ml, which corresponded to fill heights of 0.5 cm, 1.0 cm, and 1.9 cm, respectively, as determined by a caliper (Table 1).

<table>
<thead>
<tr>
<th>Bag Fill Height</th>
<th>Day 0</th>
<th>Day 5</th>
<th>Day 7</th>
<th>Day 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 cm (0.5 cm)</td>
<td>25 mL</td>
<td>50 mL</td>
<td>100 mL</td>
<td>500 mL</td>
</tr>
<tr>
<td>1 cm (1.0 cm)</td>
<td>50 mL</td>
<td>100 mL</td>
<td>200 mL</td>
<td>500 mL</td>
</tr>
<tr>
<td>&gt;1 cm (1.9 cm)</td>
<td>75 mL</td>
<td>150 mL</td>
<td>300 mL</td>
<td>500 mL</td>
</tr>
</tbody>
</table>

Table 1. Culture volumes and fill heights tested with VueLife® 197-C Bags.

T CELL CULTURE
Human CD4+/CD8+ T cells were isolated from a 1/10th Leukopak® using the StraightFrom® Leukopak® CD4/CD8 MicroBead Kit and the MultiMACS™ Celli24 Separator Plus instrument (all from Miltenyi Biotec), according to manufacturer’s recommendations. For this study, T cells were isolated from three independent healthy donors and seeded into VueLife® “C” Series Bags at a density of 1.0x10^6 cells/ml.

T cells were cultured in Gibco™ AIM V™ medium and stimulated with Gibco™ Human T-Activator CD3/CD28 DynaBeads™ at a 1:1 bead-to-cell ratio, as recommended by the manufacturer. Recombinant human IL-2 (Miltenyi Biotec) was added to the AIM V™ medium at a concentration of 30 IU/ml. Cell density was maintained throughout the duration of the experiment at ≤1x10^6 cells/mL. The culture bags were visually examined every 1-2 days, cell aggregates dispersed by manually homogenizing the culture medium, and culture samples collected to determine cell growth and medium composition.
CELL CHARACTERIZATION
Cell viability was assessed before, during and after culture using the Vi-CELL™ Cell Viability Analyzer (Beckman Coulter, Inc.). This system utilizes the Trypan Blue dye exclusion method for quantification of viable cells in the measured cell population. The surface marker profile of generated T cell subsets was assessed via flow cytometry BD LSR II flow cytometer (Becton, Dickinson and Company).

RESULTS
T cell expansion was comparable in VueLife® 197-C Series Bags for all tested heights (0.5 cm, 1.0 cm and 1.9 cm) and fill volumes (Figure 1). The different fill volumes did not significantly affect T cell proliferation rates, produced cell numbers, cell viability levels and cell phenotype (Figures 2-4).

Figure 1. T cell expansion in 197-C bags.

A. T cells were expanded at defined heights (<1cm, 1.0cm and >1cm) in VueLife® bags. Culture volumes were expanded using culture clamps to maintain cell concentration at 1x106 cells/ml. Cell proliferation was equal in all tested conditions.

B. Once T cell expansion in Figure 1A had reached the maximum defined fill volume at day 9 (100, 200 and 300 ml, respectively), cell expansion was continued to maximum fill volume of the bag (500ml) from days 9-12. T cells showed strong proliferation in the final 48 hours of culture. Shown are mean and SD values of n=3 experiments.

Figure 2. T cell viability during expansion in VueLife® 197-C Series Bags.

A. T cell viability during expansion at defined heights (<1 cm, 1.0 cm and >1 cm) in VueLife® bags. Cell viability remained at steady levels in cultures at all tested fill heights.

B. Cell viability during final culture phase at maximum fill volume (500 ml) remained at constant levels. Shown are mean and SD values of n=3 experiments.

T cell morphology and phenotype showed the expected cell shape and surface marker expression profile (Figure 3 and 4) when comparing the range of fill volumes in 197-C bags. T cell aggregates were manually dispersed during culture by gentle agitation of the cells.

Figure 3. T cell morphology during expansion in VueLife® 197-C Series bags.

T cell morphology showed characteristic cell shape and aggregation during culture in 197-C bags.
T cell surface marker expression levels were analyzed by flow cytometry before, during and after culture in 197-C bags filled to different heights. Shown are percent positive cells relative to unstained controls. Shown are mean and SD values of n=3 independent experiments.

T cell expansion was most efficient with a starting culture volume of 25 ml when compared with cultures started with 50 and 75 ml. Customers can thus initiate their cultures with lower than previously recommended fill volumes and heights and achieve the same culture production output.

The use of cryopreserved cells as starting material for this study also resulted in comparable T cell production, viability and surface marker expression profile within the tested fill heights (data not shown).

CONCLUSION
Saint-Gobain’s VueLife® “C” Series Bags support efficient expansion of human CD4+/CD8+ T cells.

T cell expansion rates remain constant at all tested fill volumes and heights, when maintained at a concentration of 1x10⁶ cells/ml during expansion in 197-C bags.

Comparable T cell proliferation rates, viability and T cell phenotype could be achieved in VueLife® 197-C Series Bags within the tested fill range.