



# CELLine Technical Report I

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CELLine Overview • Application: Introduction

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The CELLine culture devices are based on a compartmentalization approach. Cells are cultured in a cell compartment separated from nutrient medium by a semi-permeable membrane. Cells in the cell compartment are maintained in a small volume and supported by a larger volume of nutrient medium contained in the nutrient medium reservoir. Successful culture in CELLine devices require manipulation of both the cell compartment contents and the nutrient medium. Attributes such as high cell density, reduced serum use, ease of handling are available to the user through this compartmentalization approach. The following overview is provided to highlight certain features and functions of the CELLine devices to assist the user in development of individual and varied applications.

**High cell density:** Cells can be cultured at high density within the cell compartment of the CELLine. In comparison to traditional static culture, the number of cells per milliliter, which can be maintained within the cell compartment of the CELLine is much higher. For example, hybridoma cells, lymphocytes and leukemic cells can reach concentrations which are 20 to 30 fold greater when compared to growth in static vessels. This provides benefits to the user by providing increased concentrations of secreted effector molecules, high antibody concentrations, and unique cell culture environments not available in traditional low density culture.

The separation of the cells from the nutrient medium by a semi-permeable membrane allows for trapping or retaining cell secreted factors and the ability to conserve the use of exogenous culture factors. The 10,000 MWCO semi-permeable membrane allows the retention of many cytokines which are approximately 10,000 MW or larger. For instance, IL-2 provided only to the cell compartment exerts full biological activity on the cells in culture. Conversely, secreted cytokines produced within the cell compartment can reach levels not normally associated with traditional static culture. Conditioned medium can be produced with significantly different levels of effector molecules in the CELLine than in traditional static culture.

For production of monoclonal antibodies the benefits of the compartmentalized strategy provide cost savings and labor savings. Hybridoma cells can be cultivated at high density within the cell compartment of the CELLine producing highly concentrated titers of antibody within the cell compartment volume. Antibody titers in excess of 1 mg/ml are routinely achieved. Concentrated antibody in small supernatant volumes can be diluted and used directly or concentrated supernatant can be applied directly to affinity purification columns.

Importantly, the consumption of serum can be significantly reduced by providing serum only to the cell culture compartment and eliminating serum from the nutrient medium. A 1 liter culture in the CELLine consumes only milliliters of serum in contrast to hundreds of milliliters of serum which would normally be consumed in static culture. These savings in serum cost quickly accumulate as the duration of culture increases. An added benefit, is removal of interference from serum proteins during antibody purification, as the product is obtained at mg/ml concentrations in a 10% serum supernatant. This is in marked contrast to concentrating serum components in conjunction with the desired antibody during processing leading to difficulties in purification and contamination of antibody preparations with non specific immunoglobulin molecules and other serum proteins. Use of serum to generate highly concentrated supernatant of antibody molecules is not as problematic as down stream concentration steps are eliminated.

**Cell compartment:** Understanding the concept of a viable cell capacity for the cell compartment is important in the operation of the CELLine for maximum performance. The cell compartment in the CELLine has an upper limit to the number of viable cells which can be maintained within it. This is termed cell compartment viable cell capacity. As the number of viable cells within the cell compartment increases, the consumption of metabolic substrates and accumulation of metabolic byproducts also increases. Diffusion across the semi permeable membrane begins to become

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limiting when the viable cell numbers reach the viable cell capacity in the cell compartment even when a maximum diffusion gradient is provided across the semipermeable membrane. Importantly, cell proliferation does not cease, as shown in [Fig. 1](#), when viable cell capacity has been reached. Cells continue to proliferate within the cell compartment after viable cell capacity is reached. Therefore, at viable cell capacity, the number of viable cells no longer increases however total cell numbers do continue to increase. This can lead to an accumulation of very high numbers of total cells. Splitting back the cell compartment influences the ratio of total and viable cells. Splitting back the culture when capacity of the cell compartment is reached will maintain high cell viability. Splitting back several days after capacity is reached does not lead to loss of viable cells but can lead to increased product concentration and increased total cell numbers.

The handling strategy for the CELLine should be based on the needs of the operator. For high percent viability it is important to split the cell compartment back when cells reach viable cell compartment capacity. This prevents accumulation of non viable cells. For higher total cell concentrations and increased product concentrations less frequent splitting of the culture is acceptable. The ratio at which the cell compartment is split during harvest will determine the time required for cells to return to maximum viable cell capacity. Cultures split back two fold, will return to maximum viable cell numbers sooner than cultures split back 4 fold. To provide extended durations between handling, the cell compartment can be split further than 50%. Results obtained with the CELLine will be dependent upon the strategy used in manipulating the cell compartment contents and in the exchange of nutrient medium as discussed below.

In summary, the cell compartment can be treated as a standard tissue culture flask except that cells are present at high concentrations. Splitting back cells in the cell compartment provides control over cell numbers and percentages of viable cells. When cells have reached the maximum capacity of the cell compartment, proliferation continues and accumulation of total cells will take place.

**Nutrient Medium:** The rate of nutrient medium exchange also impacts culture results. Diffusion across the semi-permeable membrane is driven by concentration gra-

dients established between the nutrient medium and the cell compartment medium. Glucose flux across the semi-permeable membrane is shown in [Fig. 2](#). As nutrient medium is depleted, the driving force for solute diffusion across the semi-permeable membrane is also reduced. A decrease in viable cell mass is associated with depleted nutrient medium and accumulation of metabolic byproducts.

The nutrient medium does not change color as significantly as it does in static culture flasks. The direct gas exchange across the bottom of the cell compartment reduces the accumulation of acid within the nutrient medium. Color change of nutrient medium can be used as an indicator of metabolic activity but is not as accurate for assessing when nutrient medium should be exchanged. Tracking cell numbers is the most accurate.

As shown in [Fig. 3](#), increased nutrient medium exchange does not significantly increase cell compartment capacity. Note the maintenance of the viable cell mass and the further accumulation of total cells after viable cell capacity has been reached.

In summary, nutrient medium can be exchanged to maintain the highest possible gradient for diffusion across the semipermeable membrane, or it may be exchanged less frequently to maximize the efficiency of medium use. The user can determine which strategy is most suitable for a particular application.

**Cell inoculation:** For certain cells a relationship between cell density at inoculation and the initial rate of cell growth in the cell compartment has been observed. The cell density dependent outgrowth of a murine hybridoma cell is shown in [Fig. 4](#). When cells were inoculated at lower concentrations they took longer to reach cell compartment capacity, in comparison to cells inoculated at higher density. The responses of certain cells to low density inoculation may be due to dilution of growth promoting or conditioning effects currently not well characterized. Certain cell lines may benefit from higher initial inoculation densities. For production purposes maximum performance is attained when the CELLine is operated at or near viable cell capacity and inoculating a higher number of cells at culture initiation can lead to more rapid attainment of viable cell capacity.



**Osmotic Flux:** Water flux across the semipermeable membrane is driven by differences in protein concentrations across the membrane. When serum is not provided in the nutrient medium an oncotic gradient between the cell compartment and the nutrient medium compartment is established. This can lead to increases in the cell compartment volume during culture. The change in volume can be controlled by manipulating the oncotic gradient across the semipermeable membrane through the addition of an inexpensive protein hydrolyzate (lactalbumin) to the basal medium. In most applications the slight flux of water into the cell compartment is insignificant and can be compensated by providing slightly higher concentrations of serum or supplements to the cell compartment. As shown in [Fig. 5](#), changes in the cell compartment volume can be influenced by serum concentration differences across the semi permeable membrane, the amount of cells in the cell compartment and the duration between handling of the cell compartment.

**Cell compartment volume capacity:** The cell compartment volume is variable. As shown in [Fig. 6](#), the cell compartment can be varied significantly due to the compliance associated with the semi permeable membrane. For the CL 1000 units, a slight change in volume of the cell compartment can be induced with no significant increase in pressure. This allows the user to explore protocols with different cell compartment volumes. It is recommended that for routine use, the specified cell compartment volumes be adhered to as increased stress on the semi permeable membrane is associated with significant departure from recommended cell compartment volumes. Additionally, the impact of stretch on the semi-permeable membrane and solute diffusion is not fully characterized.

**Scale:** Results obtained in any of the INTEGRA Biosciences CELLine models can be useful in predicting results expected in the other devices. As shown in [Fig. 7](#), the cell concentrations and growth rates obtained in the small scale CL 6 WELL were also obtained in the CL 350 and CL 1000 in independent cultures of the same cell under identical conditions.

This allows experimental work to be conducted in the small scale CL 6WELL and carried directly into the larger CELLine units for scale up purposes. The scale of CELLine devices is related to the surface area of the semipermeable mem-

brane. The larger the surface area the greater the viable cell capacity. The viable cell capacity of the CL 6WELL and CL 350 are approximately 1/30 and 1/3 of the CL 1000 respectively.

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Figure 1: Growth curve example: Theoretical growth of cells within the cell compartment of the CELLine is depicted. The open circles represent viable cell numbers and plateau when the cell compartment capacity is reached. Total cells represented by open squares continue to increase throughout the culture period. Non-viable cells represented by filled circles begin to accumulate after cell compartment capacity is reached.

Figure 2: Glucose diffusion across semipermeable membrane. Tests conducted at room temperature. RPMI-1640 was placed in nutrient reservoir of individual CL 6WELL compartments. The cell compartment filled with distilled water. Cell compartment harvested at indicated times and glucose concentration determined. The driving force for glucose flux decreased with time as the cell compartment glucose concentrations increased. Flux of nutrients across the semi permeable membrane during culture will be influenced by depletion of nutrients during culture.

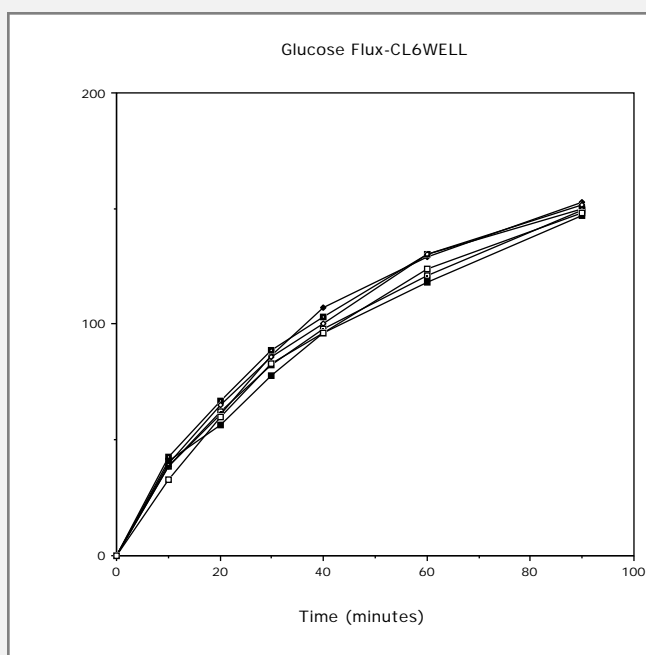
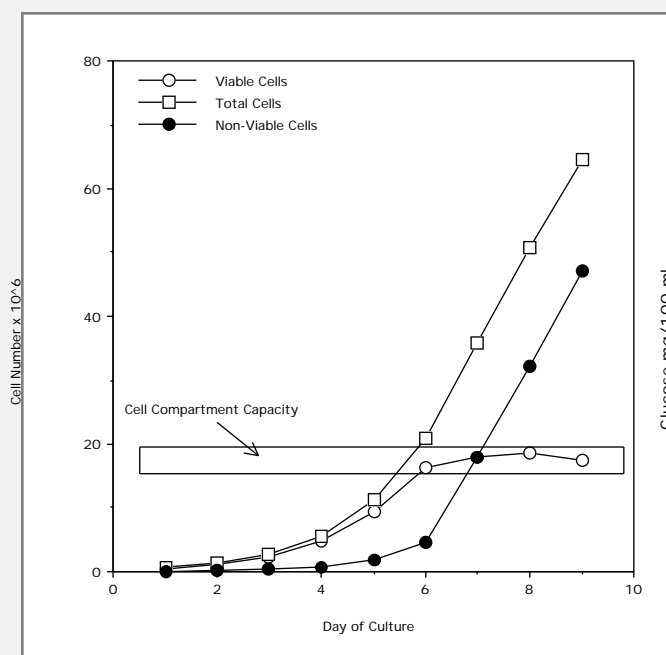


Figure 1: Cell compartment capacity

Figure 2: Glucose flux (all)



Figure 3: Growth curve of murine hybridoma cells in CL 1000. Cell compartment: 10% FBS, Nutrient compartment 0.8% FBS, .1% vitacyte. Concentrations of viable and total cells are plotted during culture. The viable cell concentration remained relatively constant after cell capacity had been reached. Total cell concentration continued to increase with culture. Note that exchange of basal medium did not significantly increase the viable cell numbers.

Figure 4: Cell outgrowth and initial cell concentration. AC.04 murine hybridoma cells were inoculated at  $0.5 \times 10^6$  and  $2.0 \times 10^6$  cells/ml into individual wells of a CL 6WELL. Cell compartment 15% FBS, nutrient compartment 0% FBS, RPMI-1640. At days indicated cells were counted and viable cell concentrations determined by trypan blue exclusion.

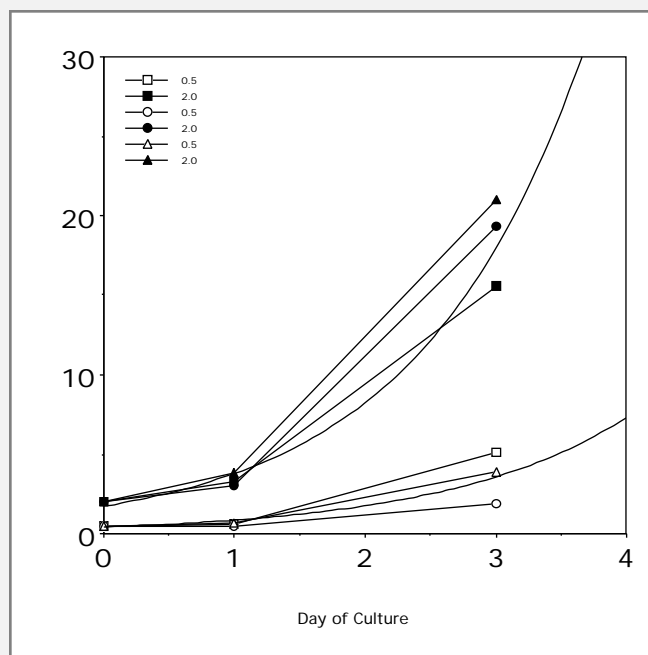
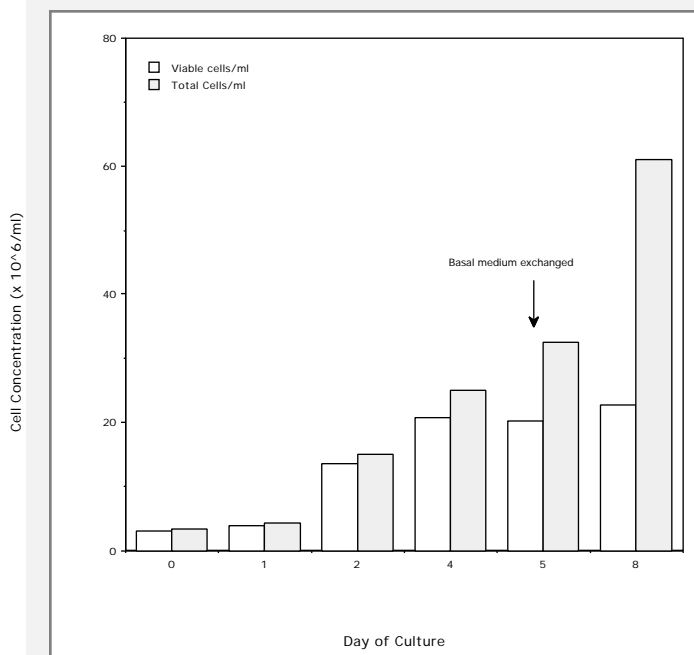


Figure 3: 10.1 Cell conc bar

Figure 4: Incoculation density

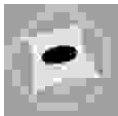


Figure 5: Osmotic water flux into cell compartment of CL 1000 and CL 350 units during culture. Cell compartment volumes are plotted at beginning and end of harvest intervals taken during culture. Cultures were conducted with cell compartment serum concentrations of 10% (0.8% nutrient compartment) and 15% (0% nutrient compartment).

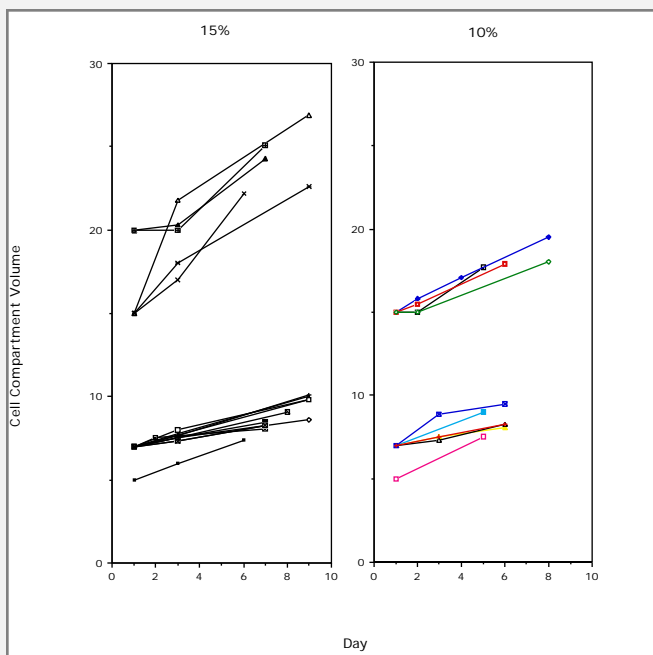


Figure 5: 10 and 15 % water flux

Figure 6: Cell compartment volume and pressure relationship. The volume of infused air into a wetted cell compartment of individual CL 1000 units is plotted versus gauge pressure. Units were tested with 250 ml of water placed atop of cell compartment. Pressure was incrementally increased in 20 sec intervals until membrane burst or developed leak.

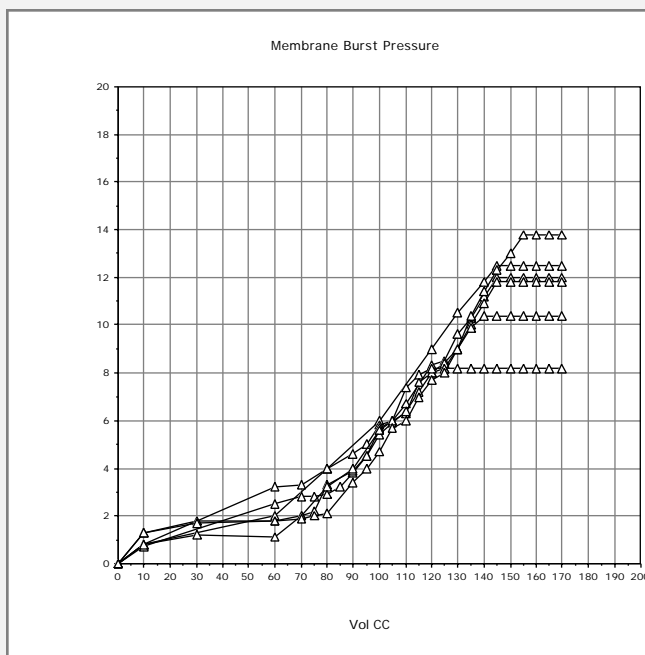


Figure 6: Volume pressure curve

Figure 7: Murine hybridoma cells cultured under identical conditions in different CELLine devices: Cell compartment 15% FBS, RPMI-1640, Nutrient compartment-0% FBS, RPMI-1640. Cell sample removed from cell compartments on day indicated and cells counts determined. Counts were obtained from a single CL 1000, CL 350 and 2 CL -6WELL units. The results from the CL 6WELL were obtained from a single well. Cultures were split back on indicated days.

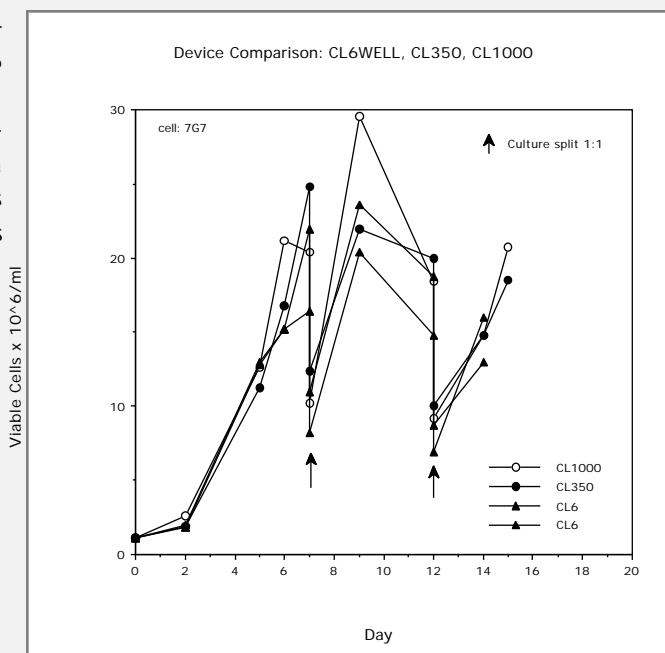


Figure 7: Device comparison