



**Project #:** CF-C-10-36-006

**Project Name:** Hybridoma culture in CellTumbler in 5 L scale

### **Purpose:**

The aim of the project is to test the CerCell CellTumbler platform using wave bags from GE Healthcare and compare and monitor temperature and growth performance for a batch culture of a hybridoma cell line.

### **Materials and Methods:**

The CerCell CellTumbler platform (Kit#13 from CerCell) was used in the tests and consisted of a twin platform with individual temperature control but common drive-unit. A Gas-Unit for air/CO<sub>2</sub> supply from GE Healthcare was used for oxygen supply and pH control. Wave bags with a working volume of 5 L were from GE Healthcare and aerated with a flow of 0.1-0.2 L/min with CO<sub>2</sub> % between 5 and 10%. Rocking speed was maintained at 22 rpm and the temperature was logged manually twice a day and maintained at  $36.8 \pm 0.2^\circ\text{C}$ .

The hybridoma 9E10 cell line (ATCC CRL-1729) expressing a monoclonal antibody against c-Myc was adapted to grow in CD Hybridoma Medium supplemented with 8 mM L-Glutamine and 100 U/mL penicillin/streptomycin ( all from Invitrogen) using standard tissue culture techniques. Following adaptation the cells were expanded in culture flasks until enough cells were available to seed a wave bag mounted on the CerCell CellTumbler platform. Growth performance was monitored by daily sampling and cell were expanded until a final culture volume of 4 L. Cell were stained with 0.4% Trypan Blue Stain and non-viable and viable cells counted in a hemocytometer under a microscope.

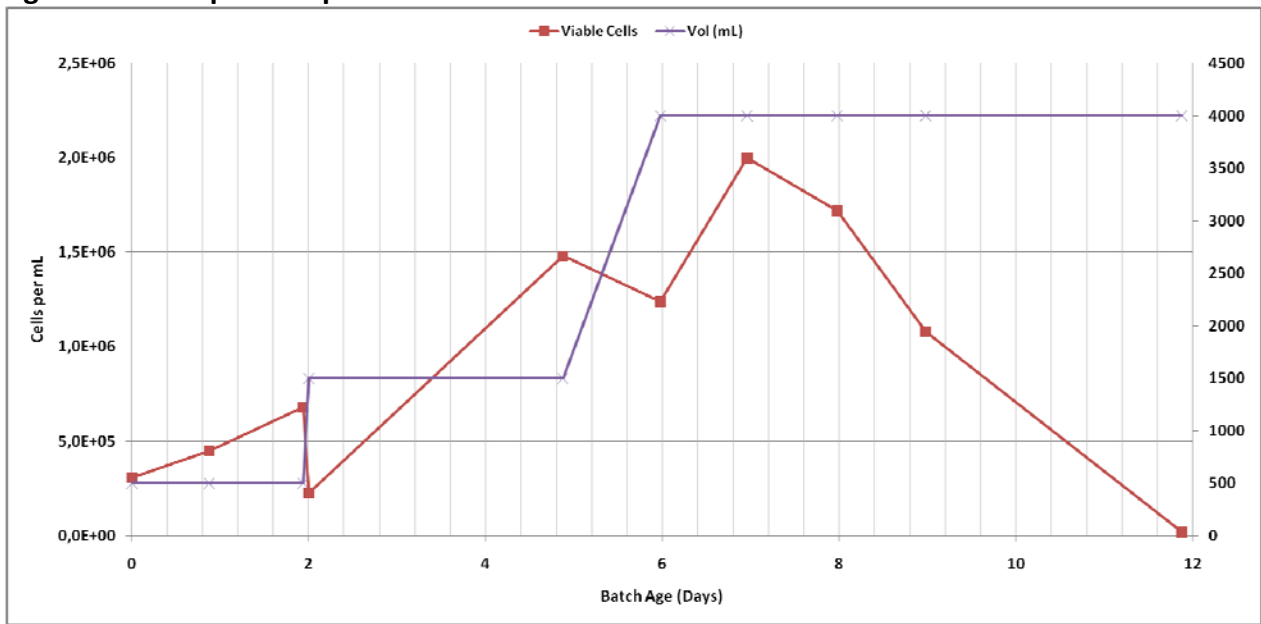
### **Results:**

Following expansion in culture flasks the hybridoma 9E10 seed culture of 100 mL was transferred into 400 mL preheated complete CD Hybridoma Medium to a final volume of 500 mL. At this point the cell viability was ~100% and had cell density of  $3.08 \times 10^5$  cells/mL. During the expansion phase the cells were kept in log-phase and were diluted during the following days to maintain the cells in the log-phase (*Figure 1*).

After 6 days the final volume of 4 L was reached after which the culture was maintained in batch mode until the viability has decreased to zero. At this point the titer is usually at its highest for this antibody without cell death affecting the product quality (data not shown).

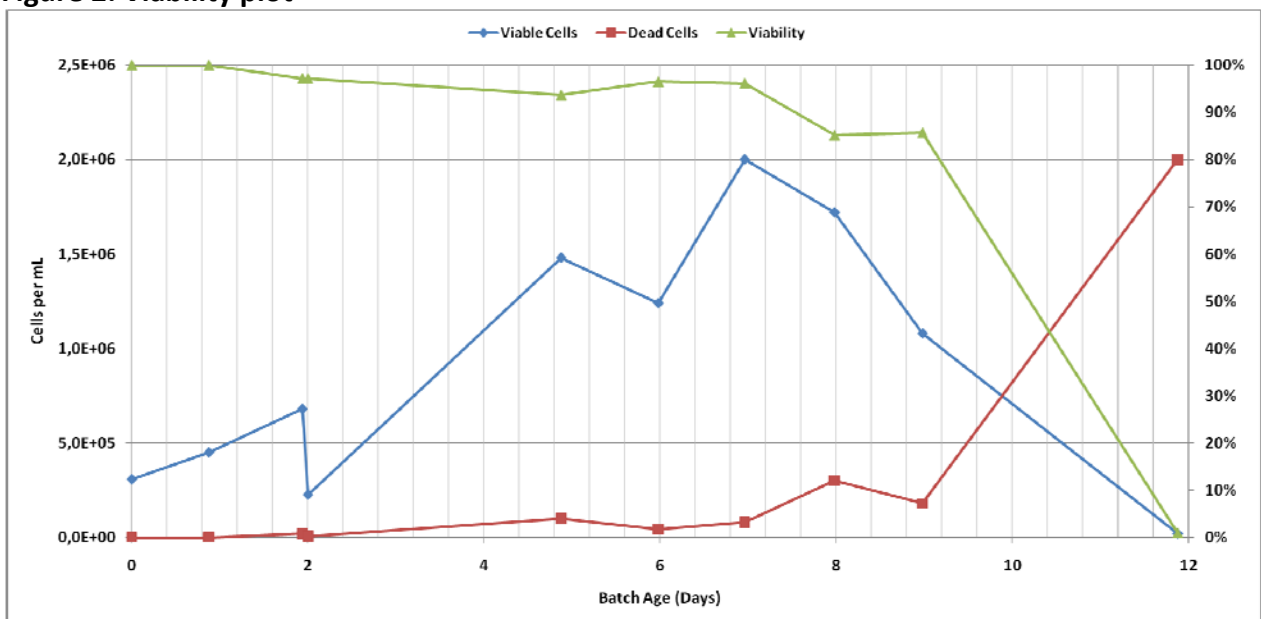


Figure 1: Cell expansion plot



The viability of the hybridoma 9E10 cell culture is maintained above 95% for 7 days where after the medium becomes exhausted and the cells begin to die. (Figure 2)

Figure 2: Viability plot



The growth data is comparable to results obtained using GE Healthcares Wave system on the same hybridoma 9E10 cell line.