



**Project #:** CellTumbler (CF-C-10-42-008); WAVE Bioreactor, historic data (CF-C-09-19-008)

**Project Name:**

Stably transfected CHO-K1 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 monitor growth performance for a batch culture of a stably transfected CHO-K1 culture line. Data from the CerCell CellTumbler platform is compared with historic data from the GE Healthcare WAVE Bioreactor System.

**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 WAVE Bioreactor Systems 2/10 (Base 2/10 EH) from GE Healthcare was used for the historic data. 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 was maintained at  $36.8 \pm 0.2^\circ\text{C}$ .

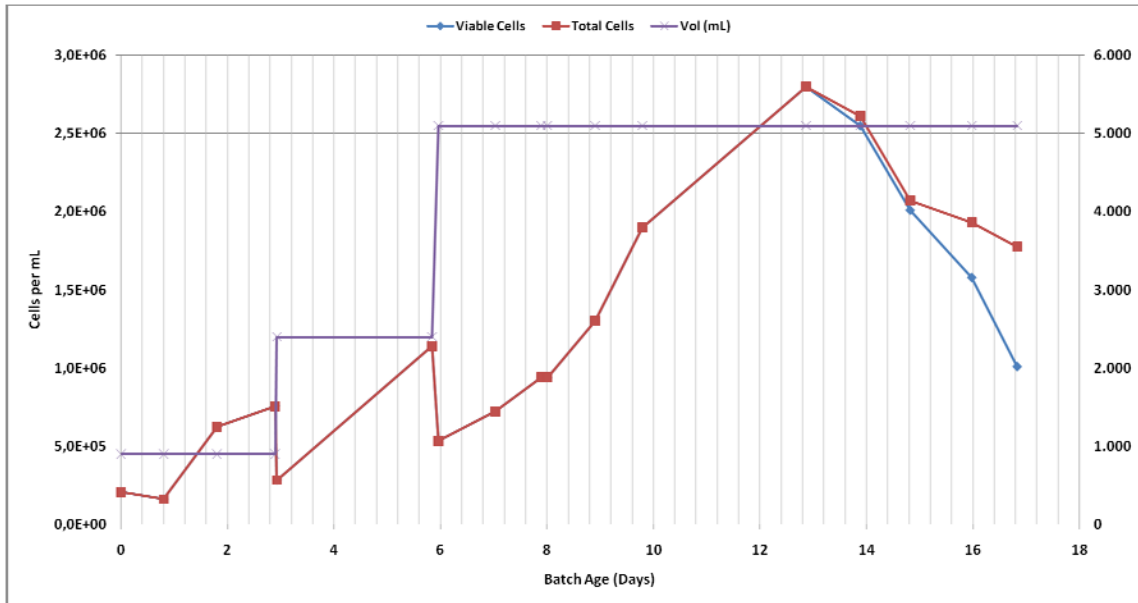
A stably transfected CHO-K1 cell line expressing a non-disclosed *in-house* human receptor fragment was adapted to grow in suspension in Hybridoma-SFM, 2 mM Glutamax; 100 U/ml penicillin/streptomycin ; 0,1 % pluronic acid ( all from Invitrogen) using standard tissue culture techniques. Following adaptation the cells were expanded in culture flasks until enough cells were available to seed a wave bag (GE Healthcare) mounted on the CerCell CellTumbler platform. Growth performance was monitored by daily sampling and cell were expanded until a final culture volume of 5.1 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 CHO-K1 seed culture of 100 mL was transferred into 800 mL preheated complete CD Hybridoma Medium to a final volume of 900 mL. At this point the cell viability was ~100% and had cell density of  $2.09 \times 10^5$  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*).

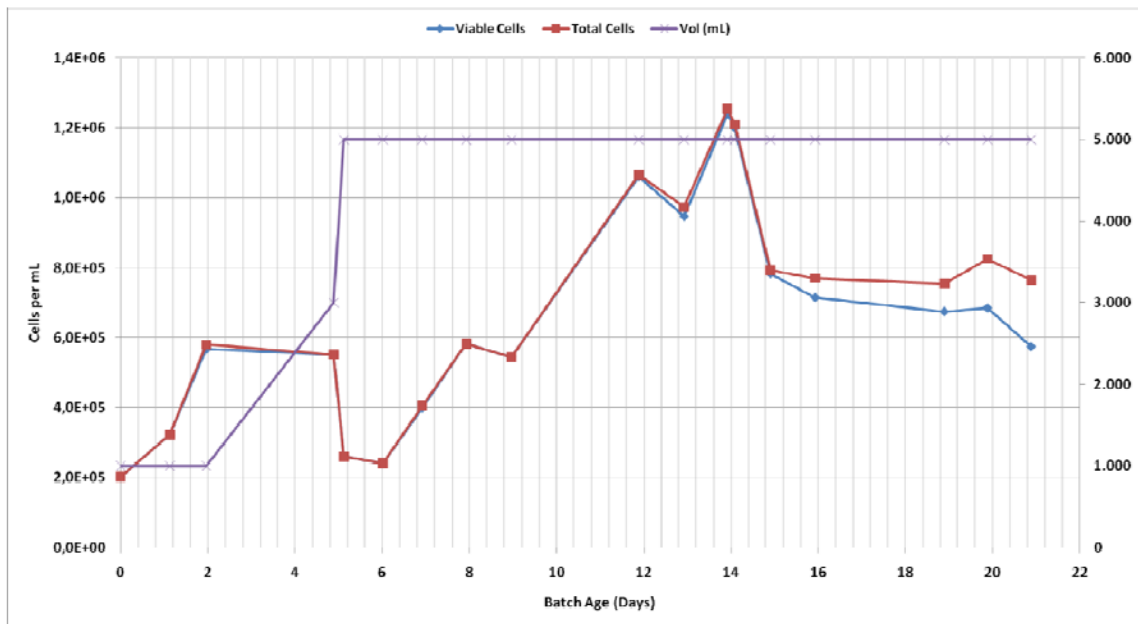
After 6 days the final volume of 5.1 L was reached after which the culture was maintained in batch mode until the viability has decreased below 60%. At this point the titer of the receptor fragment is usually at its highest without cell death affecting the product quality (data not shown).

**Figure 1: Cell expansion plot (CellTumbler)**



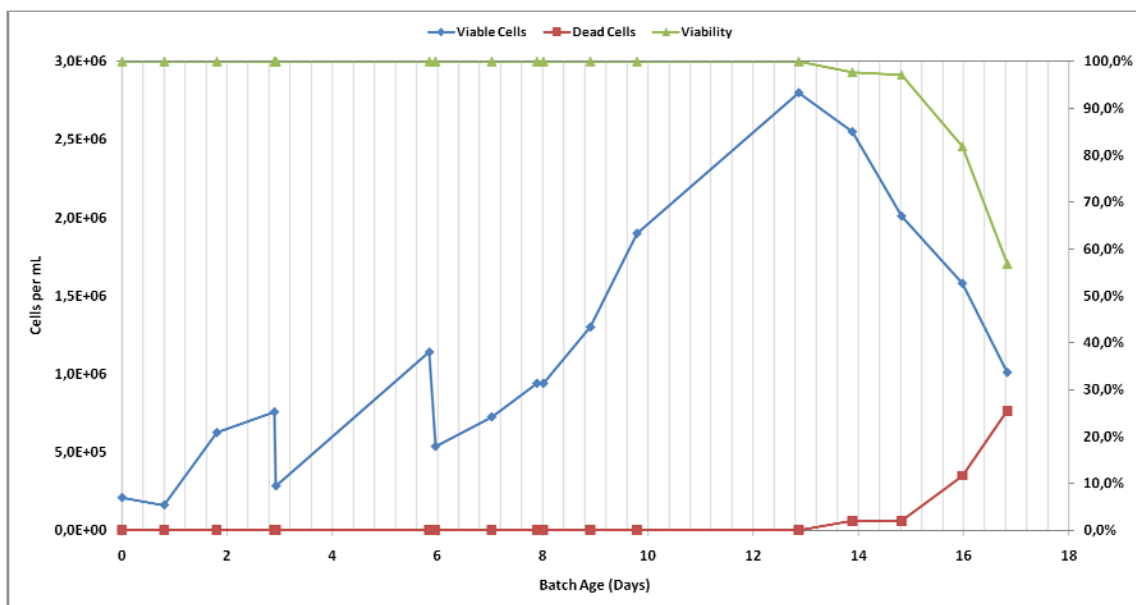
Below in figure 2 data from a similar run on a WAVE Bioreactor system from GE Healthcare using the same cell line and expansion plan. At the seeding point the cell viability was ~100% and had cell density of  $2.02 \times 10^5$  cells/mL. During the expansion phase the cells were kept in log-phase and were diluted during the following days to maintain the cells in the log-phase (*Figure 2*). However, unlike the Cell Tumbler run above a single injection of a glucose feed was done at day 14.

**Figure 2: Cell expansion plot (WAVE Bioreactor, historic data)**



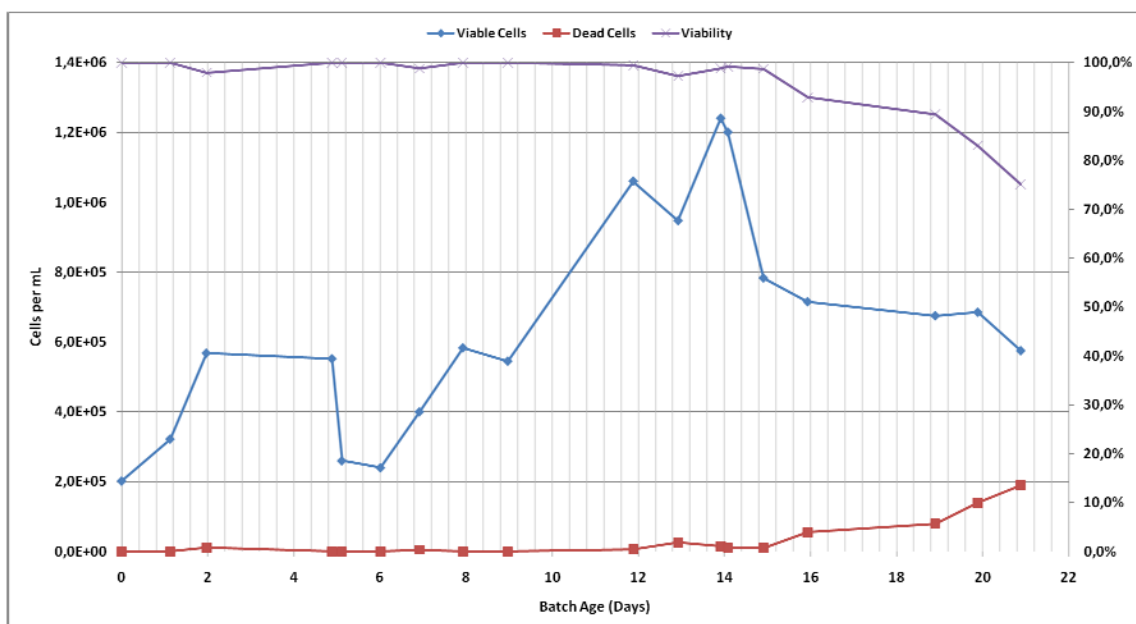
The viability of the CHO-K1 cell culture is maintained above 98% for 14 days where after the medium becomes exhausted and the cell density start to decrease. (Figure 3)

**Figure 3: Viability plot (CellTumbler)**



The viability of the WAVE bioreactor system CHO-K1 cell culture is maintained above 98% for 15 days where after the medium becomes exhausted and the cell density start to decrease. However the effect of the glucose injection at day 14 prolongs the death-phase until day 21 (Figure 4)

**Figure 4: Viability plot (WAVE Bioreactor, historic data)**



In comparison there is little difference between the two runs until day 14, after which they differ due to the addition of glucose to the WAVE bioreactor system.