Title: **CHO transient expression in wave bags**

Product: **A chimeric IgG1 antibody**

1. Aim

To examine transient expression of a chimeric IgG1 antibody using CHO cells in the CerCell CellTumbler platform using commercially available wave bags. The cultivation and expression was compared to an alternative widely used commercially available Wave system and to shaker cultures.

2. RESULTS

The transient expression using CHO was carried out in three experiments:

Experiment A

- 4 x 20 ml shaker
- 1L alternative Wave (aW) system

Experiment B

- 1L Cell Tumbler
- 1L alternative Wave (aW) system
- 1 x 20 ml shaker
- 1 x 50 ml shaker

Experiment C

- 1L Cell Tumbler
- 2 x 50 ml shaker

The results of Experiment A show that transient expression in the 1L alternative Wave system is as least as good as the shaker controls (Figure 1).

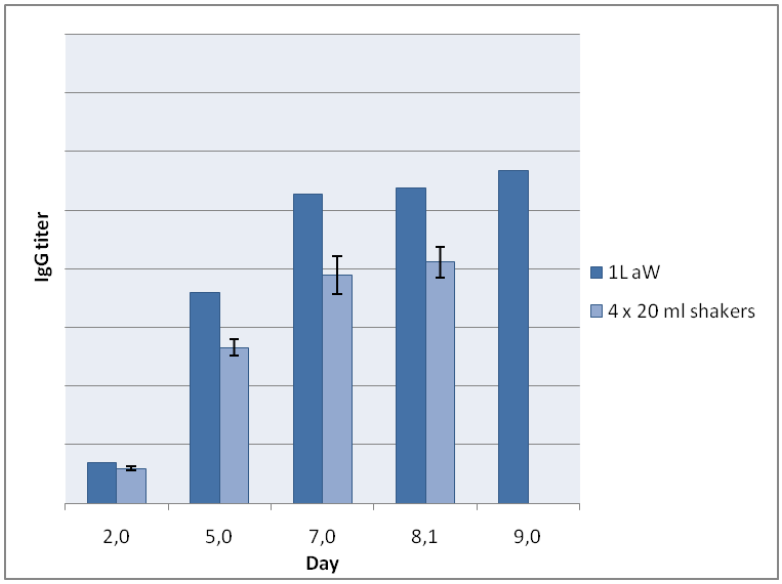

Figure 1. IgG titers obtained in experiment A in 1L alternative Wave system (aW) and shakers. For shakers +/- standard deviation is shown.

Comparison of 1L Cell Tumbler, 1L Wave and 20 and 50 ml shakers in Experiment B, show that transient expression in the 1L Cell Tumbler is at least as good as the shaker cultures (Figure 2). The 1L alternative Wave did not perform as well in this experiment but this was due to an airflow error (affecting pH) after day 3.

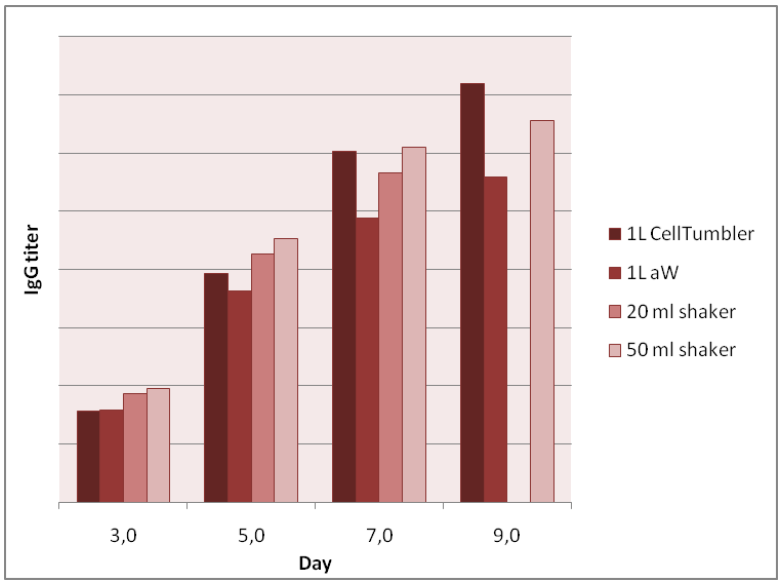

Figure 2. IgG titers obtained in experiment B in 1L Cell Tumbler, 1L alternative Wave and shakers.

Comparison of 1L Cell Tumbler and two 50 ml shakers in Experiment C show that transient expression in the 1L Cell Tumbler is at least as good as the shaker cultures (Figure 3).

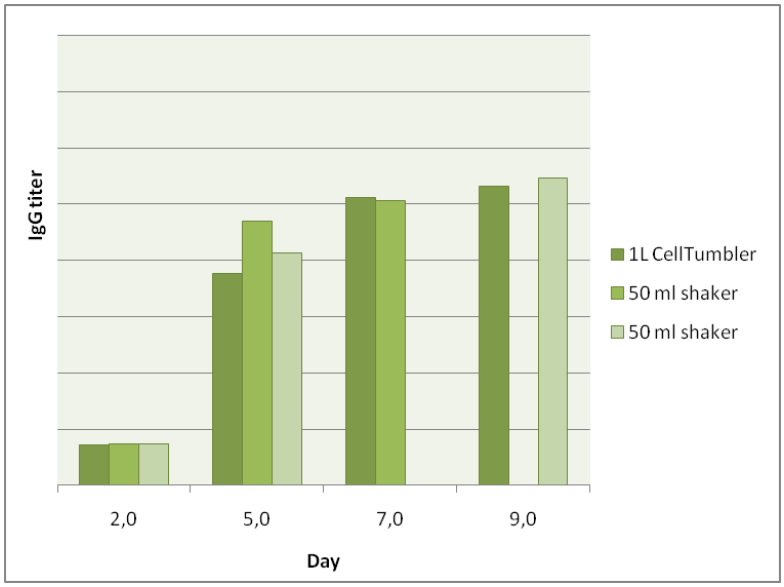

Figure 3. IgG titers obtained in experiment C in 1L Cell Tumbler and shakers.

Comparison of titers from the 3 successful cultivations in wave bag shows that the 1L Cell Tumbler is at least as good as the alternative Wave system (Figure 4).

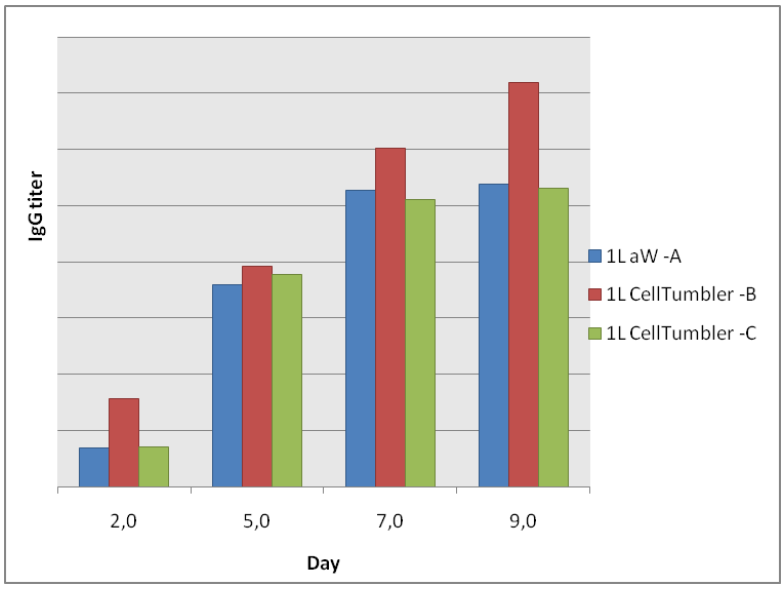

Figure 4. IgG titers obtained in the three successful cultivations in wave bag either using the alternative Wave (aW) system or the Cell Tumbler Platform.

3. PROCEDURE

3.1 Cultivation systems

CerCell's CellTumbler platform (Kit#13 on http://cercell.com/products/celltumbler/products/configurations) was used for the tests. It consists of: An aluminium frame fitted with two platforms; the Drive-Unit; The Heat-Control-Unit with independent channels for two platforms and a Gas-Unit for air/CO2 supply (Figure 5). The CellTumbler was compared to

either a conventionally available Wave bag platform or shaker flask cultures. All transient expression in wave bags were performed in 2L Cell Bags (GE Healthcare).

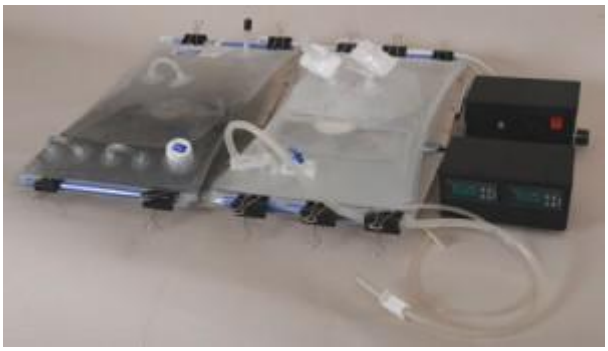

Figure 5. Cultivation systems used. CerCell's CellTumbler platform fitted with 2x5L Cell Bags. Note that the transfection experiment was performed in 1L scale in 2L cell bags.

3.2 Seed train

CHO cells were expanded in shaker flasks at 140 rpm in an incubator with 5% CO_2 at 37°C. Cells were passaged 3 times a week.

3.3 Transient expression

Transient expression was initiated by transfecting the CHO cells with plasmid encoding the IgG1 antibody and then using either shakers or wave bags for cell growth and transient antibody expression.

3.4 Cultivation settings

Table 1. Cultivation settings.

Parameter	1L alternative Wave	1L Cell Tumbler	20 ml in shaker	50 ml in shaker
Rocks pr. min	37	35	N/A	
Shaker angle	9°	9°	N/A	
Aeration (lpm)	0.1	0.1	N/A	
CO2 addition (%)	5%	5% - 0% day 5	5%	
Temperature (°C)	37°C => 32°C day 1	37°C => 32°C day 1	37°C => 32°C day 1	

Inoculum viable cell conc. target (E+06 viable cells/ml)	1.8	1.8	1.8	
Rpm	N/A	N/A	140 rpm	
Cultivation vessel	Cell Bag: CB0002L10-02		Corning 125 ml flask w. 0.2µm vent cap, 431144	Corning 250 ml flask w. 0.2µm vent cap, 431144

3.5 Sampling

Cell counts were performed on a ViCell XR cell Counter, an ABL-5 was used for analysis of CO2, offline pH and O_2, IgG1 titers were measured by a Protein A high-throughput method and a BioProfile 100plus was used for metabolite measurements.