

# Growth of Recombinant and Non-recombinant CHO-cells

## Objective

Many proteins for pharmaceutical purposes can be produced by recombinant animal cells. The most commonly used cell for this purpose is Chinese Hamster Ovary(CHO) cells. CHO cells can be cultured in large scale by established procedures and strains with different selection systems are readily available. However, depending on the type and amplification of the inserted gene, clones that exhibits growth characteristic markedly different from the mother cells usually results. This application note compares the growth characteristics of a standard CHO-K1 with a recombinant clone.

## Culture conditions

**Vessels:** 50 ml spinners(Techne).

**Microcarrier:** 2 g/l CultiSpher-G prepared according to instructions.

**Cell line:** CHO-K1(PHLS) and r-CHO.

**Agitation speed:** 45 RPM.

### Media:

**CHO-K1:** DME supplemented with 10% FBS, penicillin G(100 U/ml) and streptomycin(100 µg/ml).

**r-CHO:** a-ME supplemented with 10% dialyzed FBS, 0.5 µM Methotrexate, penicillin G(100 U/ml) and streptomycin(100 µg/ml).

pH was controlled through CO<sub>2</sub> atmosphere for both media.

## Results

Both recombinant and non-recombinant CHO-cells attached to and grew efficiently on CultiSpher-G. At maximum growth rate the doubling time was 20 hours for recombinant cells versus 12 hours for non-recombinant. After 13 days of growth, the cell yield was 35·10<sup>8</sup> cells/g dry weight CultiSpher-G which corresponds to an increase in cell number by a factor of 70 for recombinant cells. The higher growth rate for non-recombinant cells resulted in a cell yield of 30·10<sup>6</sup> cells/g dry weight CultiSpher-G after 5 days of growth. This corresponds to an increase in cell number by a factor of 30.

## Discussions

Approximate the same cell yields were obtained with both cell lines. This indicates that the available surface area is larger than that needed for the number of cells able to be supplied by oxygen and nutrients by this system. The cell concentration obtained, 6.0·10<sup>6</sup> cells/ml, have previously (Application Notes 100, 107, 108, 109, 110 and 111) been reached for CHO-K1 cells with CultiSpher-G used at 1 g/l. Especially application Note 110 shows that, without any special systems for oxygenation, CultiSpher-G should not be used at concentrations above 1 g/l.

The recombinant cells are grown under constant selection pressure by methotrexate to retain the amplified gene copies through cell division. This selection pressure imposes stress on the cells which results in almost twice the doubling times of non-recombinant cells.

