

# High cell density cultivation of PER.C6<sup>®</sup> cells in the CELL-tainer<sup>®</sup> single use bioreactor

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Cultivation of PER.C6<sup>®</sup> cells offers the potential to obtain high cell densities and, consequently, product titers.

For the supply of clinical and commercial grade material, a PER.C6<sup>®</sup> fed-batch platform process is available, that is capable of delivering up to 8 g/L IgG, in 250 L (single use) stirred bioreactors, thereby supplying up to 2 kg of crude product per batch.

This study focuses on technologies for straight forward and fast track supply of representative product (10 – 100 g) for pre-clinical applications and downstream processing development. Objective is to downscale the described PER.C6<sup>®</sup> fed-batch platform process in rocking-type bioreactors, which require limited operator skills and infrastructure. As such the process should be suitable to be performed in the cell line generation labs, using stable clones or even stable pools.

When rocking-type bioreactors are used, the challenge is to supply sufficient oxygen and remove sufficient carbon dioxide to support cell densities of 20 – 25 x 10<sup>6</sup> cells/ml as achieved in the PER.C6<sup>®</sup> fed-batch platform process.

For this reason a second generation rocking-type bioreactor, the CELL-tainer<sup>®</sup> was selected. Due to the very high mass transfer capabilities, with k<sub>la</sub> values of 170 hr<sup>-1</sup> and higher, the CELL-tainer<sup>®</sup> single use bioreactor should be able to support high PER.C6<sup>®</sup> biomass concentrations.

The results obtained in this study show that growth and productivity trends were similar to those achieved in stirred bioreactors. Furthermore it was shown that the required mass transfer could be attained by adjustments of the two-dimensional rocking motion of the bag only. Therefore, no addition of oxygen or means to remove carbon dioxide were required for oxygenation, inflating air with 5% CO<sub>2</sub> through the headspace is sufficient.

In conclusion, a simple process is developed, easy to install and to perform without the need of a complex infrastructure. Such a process makes it possible to obtain crude product yields of 60 – 80 g of IgG per batch in a 10 L rocking-type single use bioreactor.