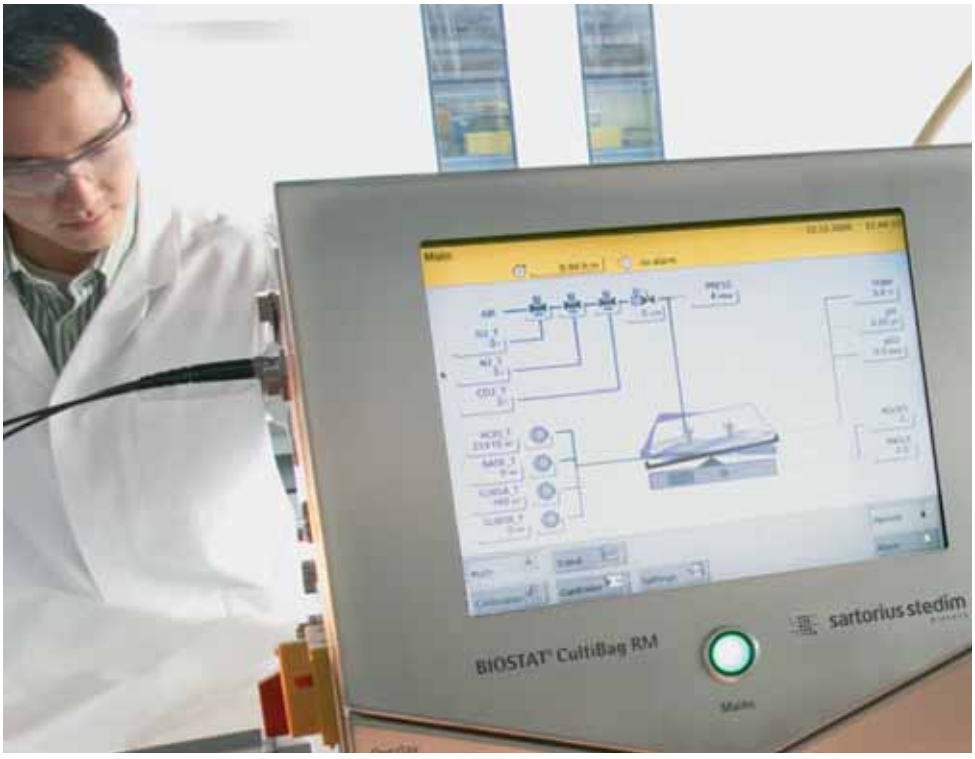
sartorius stedim biotech

BIOSTAT® CultiBag RM
Culturing Convenience



Application Note

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Cultivation of PER.C6® cells in the single use bioreactor BIOSTAT® CultiBag RM for the production of monoclonal anti-EpCAM antibodies.

turning science into solutions

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1 Background

The BIOSTAT® CultiBag RM (figure 1) is the most advanced single use bioreactor using rocking motion technology. The pillow shaped cultivation chamber is rocked back and forth, creating waves which provide mixing with low shear. The liquid surface is constantly renewed, thereby enabling efficient mass transfer between head space and media. The cultivation chamber itself is a single use bag composed of a multilayer film with pharmaceutical grade ethyl vinyl acetate (EVA) as the contact layer.

Single-use bags reduce validation costs, remove the need for cleaning, sterilizing, and provide stress free convenient culturing. A comprehensive validation guide and extractables report are offered for the bags. CultiBags with available cultivation volumes of 0.1 – 300 L are suitable for R&D, process development, seed train and production.

The BIOSTAT® CultiBag RM features full process automation using optical probes for pH and DO measurement. The control system comprises an easy to use touch screen control system with integrated measurement and control hardware, pumps, temperature and gassing systems. Easy to use, it is applicable to all cell types, including mammalian cells, plant cells, insect cells and microbial cells.

Figure1: BIOSTAT® CultiBag RM 20 optical single use bioreactor system.

The PER.C6® cell line is derived from a single, embryonic human retinoblast, which was purposely immortalized using adenoviral DNA. PER.C6® cells can replicate indefinitely in cell suspension under serum-free conditions. The cell line is commonly used in modern biopharmaceutical operations such as large-scale manufacturing of a multitude of biopharmaceuticals such as vaccines and therapeutic proteins including antibodies.

In this application note, we report the successful cultivation of PER.C6® in the single-use bioreactor BIOSTAT® CultiBag RM. We used chemically defined, serum-free media for the purpose of producing a high titer of monoclonal antibody in batch and simple fed-batch regime. The growth of the PER.C6® cells in the CultiBag RM was compared to growth in single use shaker flasks.

2 Material

- Single-Use Bioreactor: Sartorius Stedim Biotech BIOSTAT® CultiBag RM 20 optical
- CultiBag RM 2L optical (Sartorius Stedim Biotech) single use bags. Maximum working volume 1 L
- Shaker Flask (Corning, 250 mL Erlenmeyerflask)
- CO_2-incubation shaker (Sartorius Stedim Biotech, Certomat CT plus)
- Medium: CDM4PERMAb (HyClone), 4 mM L-glutamine (Lonza), containing Pluronic F-68
- Guava PCA- system (Guava Technologies)
- PER.C6® cell line expressing monoclonal anti-EpCAM antibody (Crucell)

3 Methods

3.1 Batch Cultivation

PER.C6® cells were precultured in 250 mL Erlenmeyerflask in a CO_2-incubation shaker at 37 °C, 5 % CO_2, 85 % relative humidity at 240 rpm. For cultivation in the BIOSTAT® CultiBag RM system, the single use bag was installed on the rocking platform. The bag was inflated with air, the light fiber cables for signal transmission from the optical probes were installed, and the bag was filled with 1 L of medium. The agitation was started at 13 rocks/min and an angle of 6°. Heating was switched on at 37°C. The pH controller, set to maintain a pH of 7.1, was activated. pH control was performed by the addition of CO_2 and 1 M NaOH. The DO controller, set to maintain a DO level of 40%, was activated. The DO was controlled automatically by mixing N_2, air and O_2. The sampling rate of the pH measurement was set to 30 s.

PER.C6® cells were inoculated at a cell density of 6 x 10^5 cells/mL. The process was performed over a duration of approximately 200 h. The viable cell density, viability and monoclonal antibody titer were determined daily. The titer of the monoclonal antibody was determined by ELISA using Microarrays.

3.2 Fed-Batch Cultivation

The fed batch process was started under the same conditions like described for the batch process, except that the bag was filled with 500 mL media.

PER.C6® cells were inoculated at a cell density of 6 × 10^5 cells/mL. After 90 h, 500 mL of medium were added to the bag, and the rocking speed was increased from 13 to 16 rpm. The process was performed over a duration of approximately 260 h (11 days). Temperature, pH and DO were controlled like described for the batch process.

The viable cell density, viability and monoclonal antibody titer were determined daily. The titer of the monoclonal antibody was determined by ELISA using Microarrays.

3.3 Shaker Flask Cultivation

For cultivation in Erlenmeyerflasks, 250 mL-shaker flasks were filled with 50 mL of medium. The agitation was started at 240 rpm and an amplitude of 2.5 cm. Heating was switched on at 37°C. The CO_2-incubation shaker was set to 5 % CO_2 and 85 % relative humidity.

PER.C6® cells were inoculated at a cell density of 1 × 10^6 cells/mL. The process was performed over a duration of approximately 170 h. The viable cell density, viability and monoclonal antibody titer were determined daily. The titer of the monoclonal antibody was determined by ELISA using Microarrays.

4 Results

In the control experiment with the 250 mL-Erlenmeyerflask, the viable cell density during the batch-cultivation reached 7.6 × 10^6 cells/mL (figure 2).

In the controlled CultiBag RM bioreactor, the cells showed a better growth performance. The viable cell density and the trend of monoclonal antibody production during the batch-cultivation in the Cultibag RM 2 L optical is described in figure 3. Here, the cell density increased to a maximum of 12 × 10^6 cells/mL within 186 h.
In figure 4, the viable cell density and the production of monoclonal antibody during a fed-batch cultivation in the Cultibag RM 2 L optical is shown. Both the viable cell density and the antibody titer show a drop after 90 h. This is a result of the dilution by the addition of 500 mL media to the cell suspension. The viable cell density reached a maximum of 11.6 × 10^6 cells/mL within 212 h which corresponds to the cell density of the batch-cultivation. In contrast, the antibody concentration increased up to 1206 µg/mL, which is almost three times higher than during the batch-cultivation. This could be explained by the adaptation of the cells to the cultivation conditions, so that the addition of further nutrients resulted immediately in growth and antibody production, hence in higher volumetric productivity.
In batch as well as fed batch cultivation, the addition of oxygen to the culture was not necessary. The oxygen requirements of the culture could be fulfilled by gassing with air only and using only very moderate rocking speeds. Thus, it can be concluded that the bioreactor provides enough oxygen transfer capacity in order to support much higher cell densities.

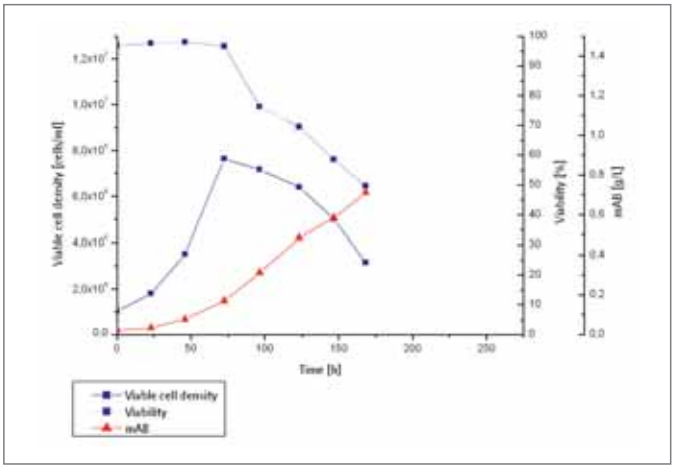

Figure 2: Batch cultivation of PER.C6® EpCAM cells in Erlenmeyerflask 250 mL at 37 °C, 5 % CO_2 and 85 % relative humidity over 170 h. Samples were collected daily and analyzed for viable cell number, cell viability and antibody titer.

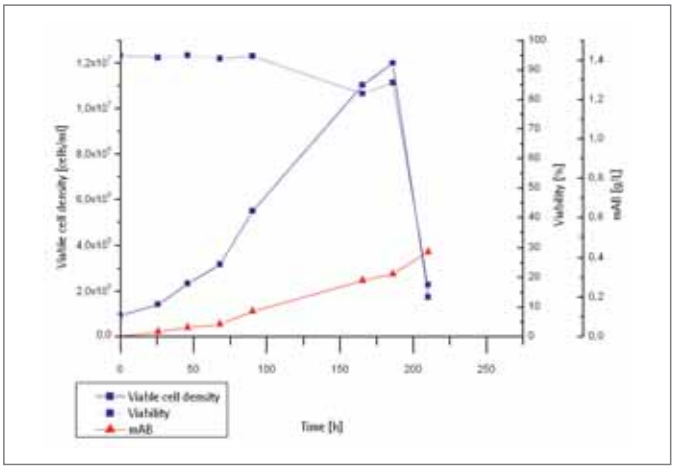

Figure 3: Batch cultivation of PER.C6® EpCAM cells in CultiBag 2 L optical at 37 °C, pO_2-control, pH-control and 13 rpm over 210 h. Samples were collected daily and analyzed for viable cell number, cell viability and antibody titer.

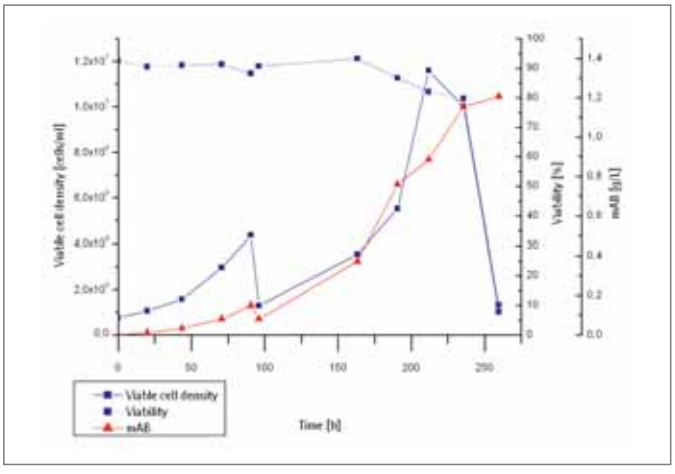

Figure 4: Fed-Batch cultivation of PER.C6® EpCAM cells in CultiBag 2 L optical at 37 °C, pO_2-control, pH-control and 13 to 16 rpm over 260 h. Samples were collected daily and analyzed for viable cell number, cell viability and antibody titer.

5 Conclusion

The BIOSTAT® CultiBag RM is the ideal tool for cutting edge applications such as monoclonal antibody production in serum free, chemically defined media. In this application note, we demonstrate that high cell densities and antibody titres could be obtained in both batch and simple fed-batch mode. Even without any optimization, antibody titres of more than 1 g/L were easily obtained.

Every part, including the sensors for pH and DO, that is in contact with product is designed as disposable, therefore removing the need for cleaning validation, keeping maintenance to a minimum and providing maximum operator safety. The BIOSTAT® CultiBag RM is a safe, reliable and easy to use tool for the cultivation of all kinds of organisms. With the available comprehensive validation guide and extractable analysis, in conjunction with full qualification and validation support including FAT and SAT, the BIOSTAT® CultiBag RM is perfectly suited for use in a GMP regulated environment.

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