CERTOMAT® CTplus: a purpose-built incubator shaker for mammalian cell cultures

Precise temperature control, stable CO₂ concentration and a humidified atmosphere are essential for long-term cultivation of mammalian cells. This could not be achieved until now for agitated cultures. The CERTOMAT®CTplus ensures optimal temperature control and even temperature distribution combined with a controlled humidified atmosphere.

Mammalian cells have traditionally been cultured in T flasks or similar devices in a CO₂ incubator. Basic requirements for such incubators include exact temperature control and very even temperature distribution. CO₂ must be injected and its concentration maintained between 0% and 20% (vol/vol) as set by the customer. Owing to the long incubation times required for growing mammalian cells, a humidified atmosphere is essential to reduce evaporation of media. Condensation of water must be kept to a minimum because any wet places may become a source of bacterial contamination. Should this happen, the whole unit, and especially the incubation chamber, must be cleaned and sanitized. The CERTOMAT®CTplus has been designed to enable perfect incubation conditions for cell culture. Stacking several units will help to save lab space.

With the recent development of suspension cell lines, which can also be cultivated in agitated flasks, a new need arises for a combination of an incubator shaker with the features of a CO₂ incubator. Solutions available so far combine either a CO₂ incubator with a benchtop shaker placed inside it or a bacterial incubation shaker modified for the injection of CO₂ and/or humidification. If a shaker is placed inside a CO₂ incubator, humidity and CO₂ will reduce the lifetime of its drive system. Furthermore, the heat generated by the shaker will impair temperature accuracy and stability inside the incubator. Conversely, incubator shakers supplemented with CO₂ and humidity suffer from unwanted condensation and high losses of CO₂ to the lab environment.

On the basis of these considerations, we set up special design requirements for an incubation shaker optimized for the cultivation of mammalian cells. This led us to a completely new design, the CERTOMAT®CTplus (Fig. 1).

Figure 1 | CERTOMAT®CTplus incubator shaker for mammalian cell cultures.

Cell cultivation

To assess the suitability of the new incubator shaker, we evaluated the CERTOMAT®CTplus for the cultivation of CHO ST1-C6 and Per.C6 Epcam cells.

We grew CHO ST1-C6 cells in proCHO5 medium (Lonza) supplemented with 4 mM glutamine and 1× HT (hypoxanthine/thymidine) at 37 °C, 5% CO₂ and 80% humidity, 240 r.p.m., 25 mm orbit in the CERTOMAT®CTplus. The viable cell count and the number of cells were determined once a day.

We grew Per.C6 Epcam cells in Per-Mab medium (Hyclone) supplemented with 4 mM glutamine at 37 °C, 5% CO₂ and 80% humidity, 240 r.p.m., 25 mm orbit in the CERTOMAT®CTplus.

Batch cultivation of these cell lines was performed in CultiFlask 50 disposable containers (Sartorius Stedim Biotech GmbH) with 15 ml medium and an initial cell concentration of 5×10^5 cells per milliliter. CHO ST1-C6 cells reached a maximum of 4.6×10^6 cells per milliliter in 93 hours, and EpCAM cells reached their maximum of 6.2×10^6 cells per milliliter in 118 hours (data not shown).

In a second set of experiments, we kept cultures under fed-batch conditions with the addition of 4 mM glutamine on days 3 and 5.

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Figure 2 | Cultivation of CHO-ST1-C6 and EpCAM cells at 37 °C, 5% CO₂ and 240 r.p.m. Samples were taken once a day and analyzed for cell numbers and cell viability. At days 3 and 5, 4 mM glutamine was added, which resulted in higher cell density of Per.C6 EpCAM cells.

Figure 3 | Cultivation of EpCAM cells at 37 °C, 5% CO₂ and 240 r.p.m. Samples were taken once a day and analyzed for cell numbers, cell viability and antibody concentration.

In this experiment, ST1-C6 cells achieved prolonged growth to only a slightly higher maximum than in batch culture. By contrast, Per.C6 EpCAM cells responded to the fed-batch conditions by reaching a maximum of 1×10^7 cells per milliliter in 123 hours, still at high viability (**Fig. 2**).

Finally, we seeded antibody-producing Per.C6 EpCAM cells at 1×10^6 cells ml⁻¹ in 250-ml Erlenmeyer flasks, each filled with 50 ml of medium. Under these conditions the cells reached a maximum concentration of 7.6×10^6 cells ml⁻¹. The antibody concentration in the medium, monitored by ELISA (enzyme-linked immunosorbent assay), reached its maximum when the viable cell count dropped to 3×10^6 cells per milliliter (**Fig. 3**).

Conclusions

CERTOMAT®CTplus can be successfully used for medium optimization in small agitated culture containers as well as for antibody production or preparation of starter cultures in conventional Erlenmeyer flasks. Culture conditions can be optimized to achieve excellent culture results for different cell lines.

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