

High throughput cell separation – a model for upscaling

The Karlsruhe Institute of Technology has automated protocols for high throughput separation of cells for therapeutic applications on a Freedom EVO® 200 liquid handling platform. The process maintains cell viability and is more rapid and reproducible than manual methods, enabling processing of 96 samples in four hours.



Traditional antibody-based methods of cell separation and purification are extremely costly due to the large amounts of clinical-grade antibodies required, and the difficulty in removing the antibody labels from cell membranes for subsequent clinical applications. There is also a risk of initiating undesirable cellular reactions, and some target cells lack a known cell-specific surface marker. Researchers at the Institute of Process Engineering in Life Sciences, Section IV: Department of Biomolecular Separation Engineering, Karlsruhe Institute of Technology (KIT), Germany, have developed alternative, automated cell separation methods based on aqueous two-phase systems (ATPS).

ATPS is a label-free separation technology that uses two immiscible polymer solutions – for example PEG and dextran – to provide gentle separation of cells according to their physicochemical properties. PhD student Sarah Zimmermann explained: “ATPS offers many advantages compared to antibody-based methods of cell separation; cost-effectiveness, scalability and very mild, gentle conditions for purification of cell products. Initially, we performed ATPS manually, but this is tricky because the polymer phases are extremely viscous. Manual pipetting can give rise to large errors, and data is not very reproducible. Automation was the way forward.”

The Institute, led by Professor Jürgen Hubbuch, has been using Tecan liquid handling systems for high throughput purification and formulation development of therapeutic proteins for over 10 years, and has three Freedom EVO workstations – two Freedom EVO 200s and a Freedom EVO 100. The high throughput ATPS protocols were automated on a Freedom EVO 200, using different liquid classes to optimize pipetting of extremely viscous solutions. The required concentrations of the various polymers, salts and other additives that determine the

pH of the aqueous two-phase systems is entered into an Excel® spreadsheet, and the volume of each reagent needed to prepare the final ATPS is calculated using MATLAB® (MathWorks), which also converts the data to a format readable by Freedom EVOware®.

The workdeck is then set up with the stock solutions and cells, the run started, and the Freedom EVO left to prepare the aqueous two-phase systems and pipette the different solutions into 96-well plates. After shaking, the cells are added and the plates shaken again, before settling for 30 minutes to allow phase separation. Aliquots are removed from both phases of the ATPS for dilution and staining, and analyzed on a flow cytometer. “One of the challenges we faced during process development was the accurate pipetting of small cell suspension volumes,” Sarah explained. “Since the cells sediment, we needed to implement resuspension protocols. However, because of the small diameter of the standard fixed tips, cell viability was an issue. We overcame this problem by optimizing the pipetting speed, the number of mixing cycles, the use of air gaps and the partitioning volumes, and compared the results to manual cell handling. At the end of this study, we were able to reproducibly transfer 5×10^5 cells in a volume of 13 μl with a standard deviation of less than 3 %.”

Automation has made the ATPS process faster and more reproducible, enabling systematic screening of different conditions. More samples are prepared in less time – 96 samples can be screened and analyzed in four hours – with much greater precision compared to manual methods. Volumes ranging from 10 to 990 μl are pipetted using the Freedom EVO’s Liquid Handling Arm with fixed tips, with standard deviations ranging from 1.5 % for the smallest volumes to less than 0.5 % for larger volumes, even with very viscous polymer solutions. The validated high throughput screening process has



From left to right: Jürgen Hubbuch, Sarah Gretzinger and Sarah Zimmermann in front of the Freedom EVO 200

been successfully applied to the separation of differentiated and undifferentiated cells from the HL-60 cell line, adjusting the ATPS conditions so that the cells partition differentially between the two phases.

“ATPS is a very sensitive way of separating cells according to their surface properties, offering a gentle, cost-effective and scalable approach for the purification of cell products that does not significantly influence the cell viability, differentiation state or differentiation potential of HL-60 cells.

Automation of the process on a Freedom EVO workstation has enabled us to establish high throughput screening, allowing rapid directed design of purification protocols for therapeutic cells, which has proved very successful,” concluded Sarah.

To find out more about Tecan’s cell biology solutions, visit www.tecan.com/cellbiology

To learn more about the Department of Biomolecular Separation Engineering (MAB) at KIT, visit mab.blk.kit.edu/english

“Manual pipetting can give rise to large errors, and data is not very reproducible. Automation was the way forward.”