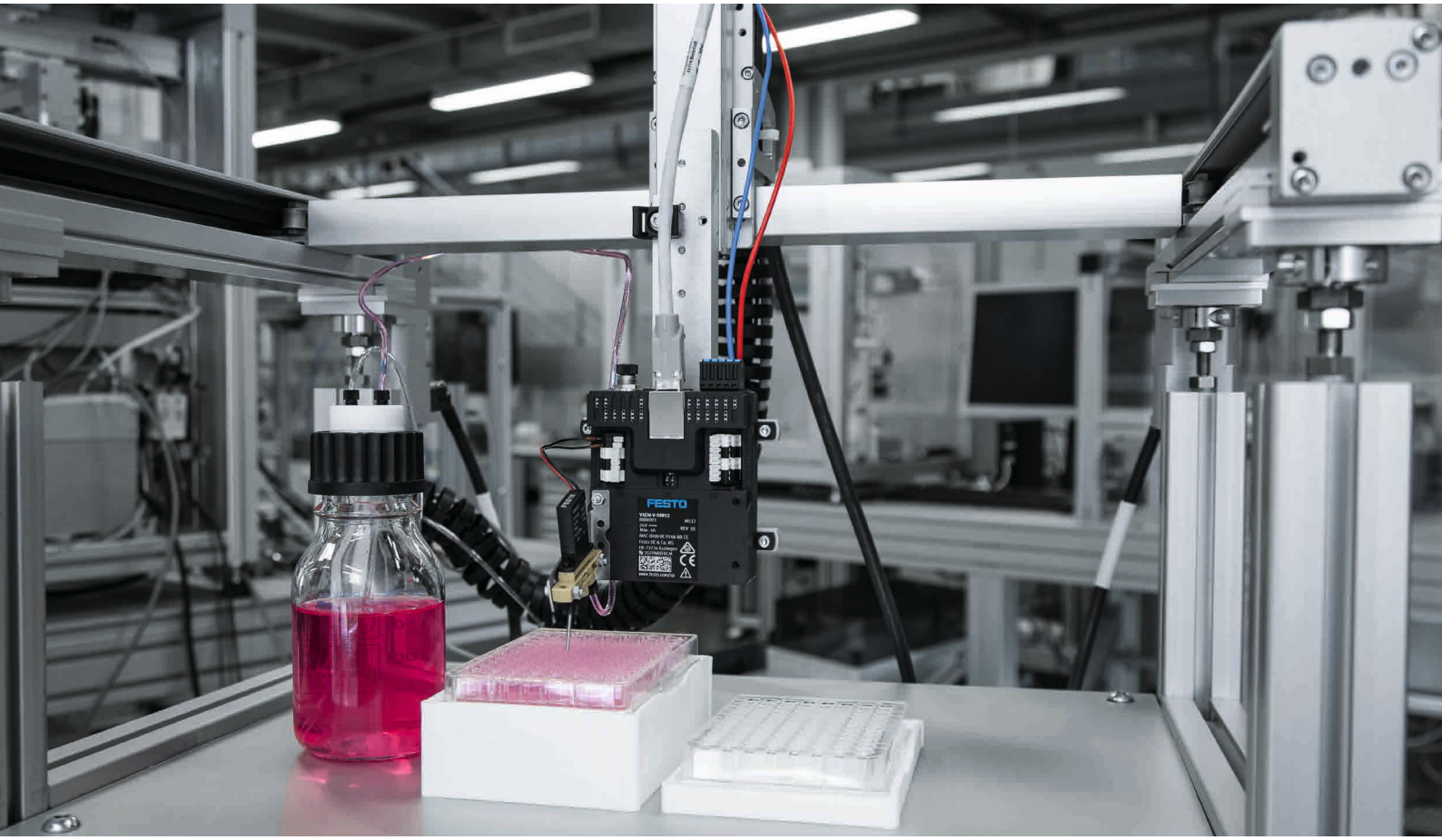
Whitepaper: A modular system for precise and automated cell dispensing

How the benefits of laboratory automation can be combined with gentle cell handling.

FESTO



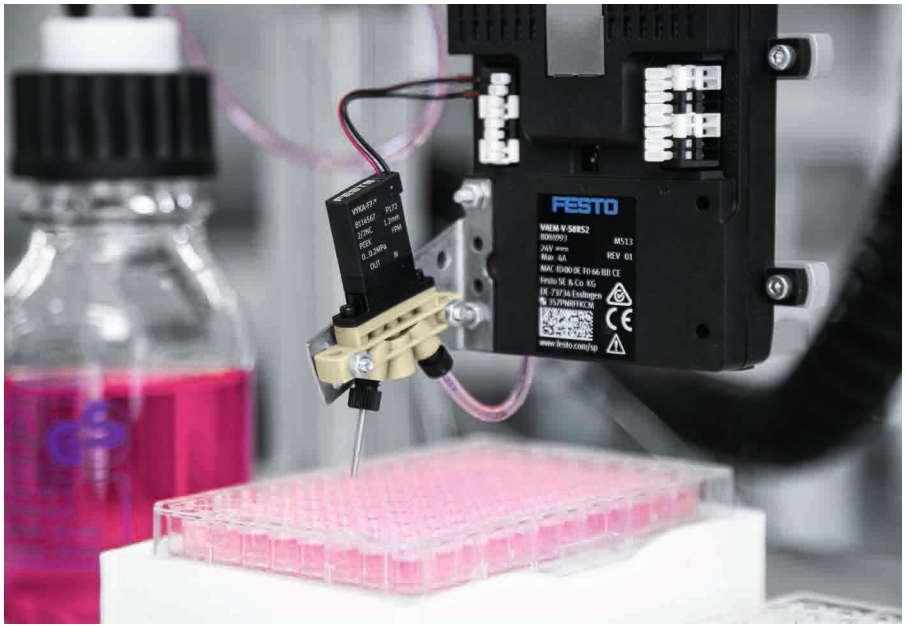
This white paper addresses the following topics:

- What are the challenges and benefits of automated dispensing of biological cells?
- How does the modular and flexible dispensing system from Festo work?
- How can the impact of an automated dispensing system on cell viability be tested?
- How high is the viability of selected cells with the Festo system compared to manual pipetting?

Executive summary

Automated liquid handling offers many laboratories many benefits, such as higher throughput, greater reproducibility, and fewer sources of error. However, dispensing biological cells poses a particular challenge. Cell viability should be affected as little as possible by the process for the results to be meaningful.

This white paper uses a selected cell line to show which products from Festo LifeTech enable automated cell dispensing. A modular and flexible system allows parameters such as pressure and valve opening times to be tailored to the cell suspension while gentle handling also minimizes the impact on viability. In the practical test, cell viability was similar to that of expert manual pipetting.



Introduction

Working with cell cultures is becoming increasingly important in laboratories and production processes. However, handling living cells or tissues is more complex than handling products made from inanimate materials, as the metabolism of the organisms and their environmental requirements have to be taken into account. That is why it is all the more important to minimize environmental interference when handling cells.

Minimal interference combined with better reproducibility, higher throughput as well as flexibility – these are just some of the reasons for using automation solutions for liquid handling. But what exactly are the benefits of an automated dispensing system compared to manual pipetting steps?

- **Time saving:** Repetitive tasks such as manual pipetting tie up scarce and expensive human resources. Automating these tasks frees up specialist staff as well as time, which can be spent on more value-adding tasks.
- **Standardization:** The work processes in manual pipetting are not completely standardized and depend on the operator. The results are therefore not always the same in terms of quality and quantity. When using manual pipettes, differences in the angle of inclination of the pipette, the positioning, as well as the aspiration and dispensing rate between different operators can lead to irregularities that affect the results.
- **Error potential:** Automated workflows reduce the susceptibility of the process to errors, as each step is carried out consistently.

- **Sterility:** To avoid contamination, it is important to ensure the work is carried out cleanly and that as few areas as possible are brought into contact with the atmosphere. An automated dispensing system provides a closed and disinfected environment for the cells and is therefore a suitable working environment.

For the reasons mentioned above, a high degree of automation in cell handling is desirable. However, the effect of any process used on cell viability should be kept to a minimum. The size and morphology of cells are always different. In addition, the cell concentration in the solutions can vary too. It is therefore important for automated liquid handling that parameters such as pressure and opening times are both adjustable and reproducible. That is how the loads acting on the cells, such as shear forces and pressure differences, can be controlled and thus minimized.

Festo offers a wide range of components for this task, which are also compatible with sensitive biological substances such as cells. There are different approaches to liquid handling in laboratory automation. In addition to pipetting, Festo also offers pressure-controlled dispensing systems. Automated pipettes are particularly suitable when different samples need to be protected against contamination or when reagents need to be added and mixed. Dispensing solutions offer benefits when the same liquid needs to be dispensed from a larger container into smaller units.

The liquid, for example a cell suspension, is dispensed directly from a large source container using a needle. This saves time and money. Process steps are reduced because, compared to pipetting, there is no need to pick up a tip or to aspirate the liquid. Reusable needles also help to save costs and make an important contribution to environmental protection.

Modular and flexible dispensing system

Since the 1950s, Festo has been known primarily for pneumatics, and has also been drawing on its extensive expertise in compressed air for the life science sector for several years. For liquid handling applications, it relies on the pressure-controlled dispensing method. This involves supplying a controlled pressure from a compressed air source to a closed container. The liquid inside is displaced by overpressure and fed to valves via tubing. A control unit precisely specifies the opening times of the valves. If required, this combination of Festo components can be installed on a Festo handling system to control the movement. The individual components are explained in more detail below.

Air preparation and pressure control

The pressure-controlled dispensing system from Festo uses compressed air to precisely and gently dispense liquids and, in particular, cell suspensions. As cells are living organisms that are very strongly affected by external conditions, the pressure in particular can be adapted to the requirements of the cell type and cell concentration.

Products such as the decentralized pressure and vacuum generator PGVA or the proportional pressure regulator VEAB are suitable for setups that have their own pressure supply. Both products are able to regulate the pressure very precisely thanks to the integrated piezo valves.

Proportional pressure regulator VEAB

- Very low energy consumption
- Extremely precise
- Integrated piezo technology
- Short switching times

Pressure and vacuum generators PGVA

- Integrated compressor
- Proportional pressure/vacuum regulation or preset fixed values
- 24 V power supply
- Easy to integrate

Controllers

To increase throughput and ensure high dispensing accuracy, the entire process – for both the air and media channels – needs to be controlled quickly, efficiently, and reliably. By rapidly controlling the valve opening times, the required amount of liquid is precisely dispensed, as long cycle times can lead to deviations in volume.

Festo has developed two products: the valve control module VAEM, which ensures very fast and precise valve control, and the media separated valve VYKA, which is ideal for the precise and rapid dispensing of cell suspensions from 1 µl with low CV values.

Valve control module VAEM

- Cycle time: 0.2 ms
- Integrated, adjustable holding current reduction
- For controlling up to 8 valves
- Graphical user interface (GUI)
- Analog and digital control

Media separated solenoid valve VYKA

- Very small dead spaces and low flow resistance
- FDA-listed materials
- Also suitable for aggressive media
- Developed according to ISO 13485

Connecting components, tubing, and needles

In order to precisely dispense the cell suspension through the needle, air and liquid must be moved in a closed system of components and tubing. This is only possible by using connectors and tubing whose material and size can be selected according to the properties of the medium flowing through them.

The needle is the last component of the system before the liquid is dispensed into the target container. The inner diameter, length, and angular position can be selected as required to precisely reach the target container.

Compatible with ethanol and cells

Fitting NLFA

Needles VAVN

Tubing PUN-H, PTFEN, PFAN

Compatibility with different liquids

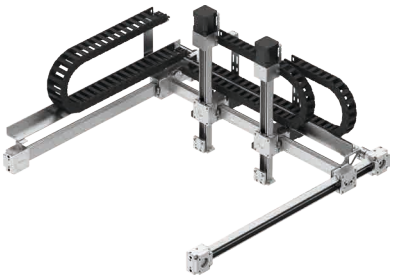
To avoid contamination, it is important that work is carried out cleanly. Two parameters play an important role here. The first is the initial condition of the components before the start of the dispensing process with the target liquid; the second is the condition of the valve interior. This will ensure that no unnecessary contamination occurs during the dispensing process.

- All the materials, from the tubing and needles to the valves, can also be disinfected with ethanol, for example.
- The cells are located in a closed system so that they are protected from external contamination. The air for the overpressure in the bottle is filtered several times.

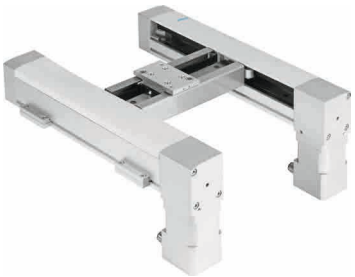
Positioning

Ensuring the dispensing process is precise not only requires the volume to be correct, but this volume also has to be correctly distributed in the target container, which is usually a microwell plate. In addition to products for dispensing liquids, the Festo portfolio also offers different components for moving and positioning, either pneumatically or electrically controlled, from fast to slow, for high to low loads.

Thanks to this modular portfolio, various processes with manual pipettes can be replaced by an automated dispensing system specially designed for the customer's application. The LifeTech portfolio from Festo offers a wide range of components and can also draw on products from industrial automation that have been tried and tested over many decades. This modular approach enables operators to find a suitable setup for the medium and volume required. This ranges from the rapid filling of 1536-well microwell plates with cell suspensions to aspirating nutrient solutions.

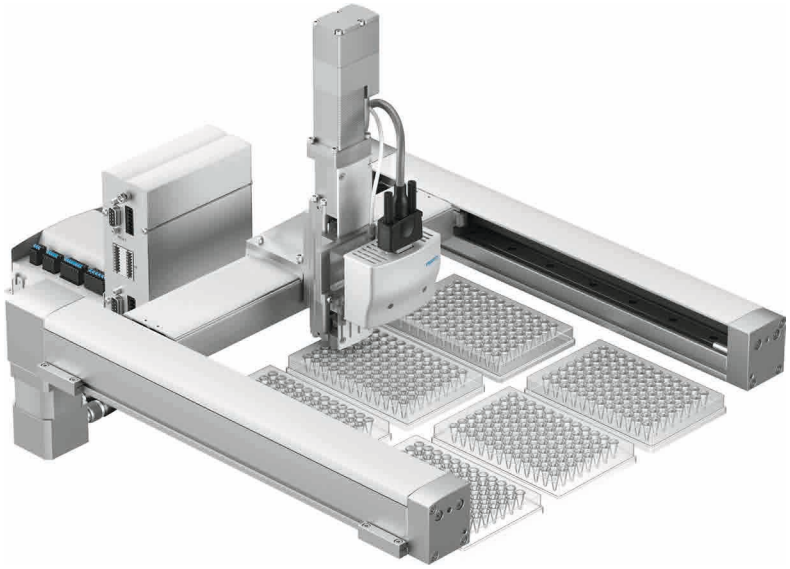


Three-dimensional gantry EXCL



Planar surface gantry EXCM

Modular and flexible dispensing system



Controllers

CECC-X

CPX terminal

CPX-IoT

VAEM

VYKA

Plug connectors, fittings, and tubing (air and liquids)

PUN-H, PTFEN, PFAN

NLFA

VAVN

NPQP

Compressed air control and preparation

MS6

MS2

LRP

VEAB

PGVA

Positioning

EXCM-30

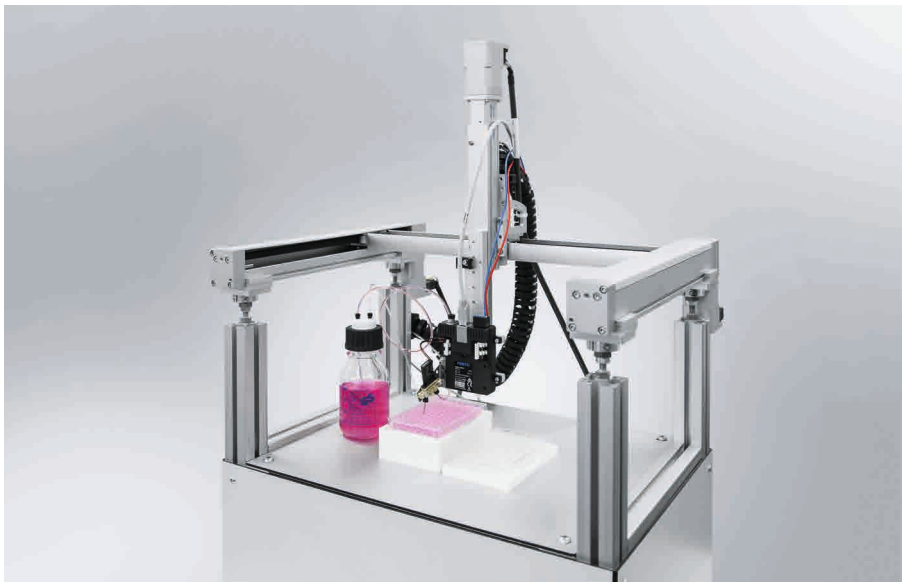
ELGC

EGSC

EMMS-ST

Practical tests for cell viability

Festo always focuses on the benefits for the end user. That is why, in collaboration with Esslingen University of Applied Sciences, Festo has tested its products for compatibility and viability with the eukaryotic cell line HEK293.



The aim of the experiment was to find out how cell viability is affected by dispensing using the Festo dispensing system compared to pipetting with a manual pipette, and to what extent the possibly "stressed" cells recover after dispensing. These effects were investigated by varying the following parameters: internal diameter, distance, and angle of inclination of the needle, pressure, and incubation time. Manual pipetting was carried out using the established Eppendorf manual pipette (volume: 20-200 µl).

Internal diameter, distance, and angle of inclination of the dispensing needle to the microwell plate

Different diameters lead to different flow resistances and thus to different shear forces, which can impair cell life. Three different diameters were therefore tested at two angles of inclination and at different distances from the microwell plate.

Internal needle diameter:
0.3, 0.6, and 1.2 mm
Angles of inclination:
0° and 15°
Distance to the target container: 3 mm to 15 mm

Pressure

Different pressures lead to different flow rates and droplet speeds, which in turn lead to different shear forces. The influence of this parameter was tested using three different pressures.

Pressure:
100, 300, and 500 mbar

Incubation period

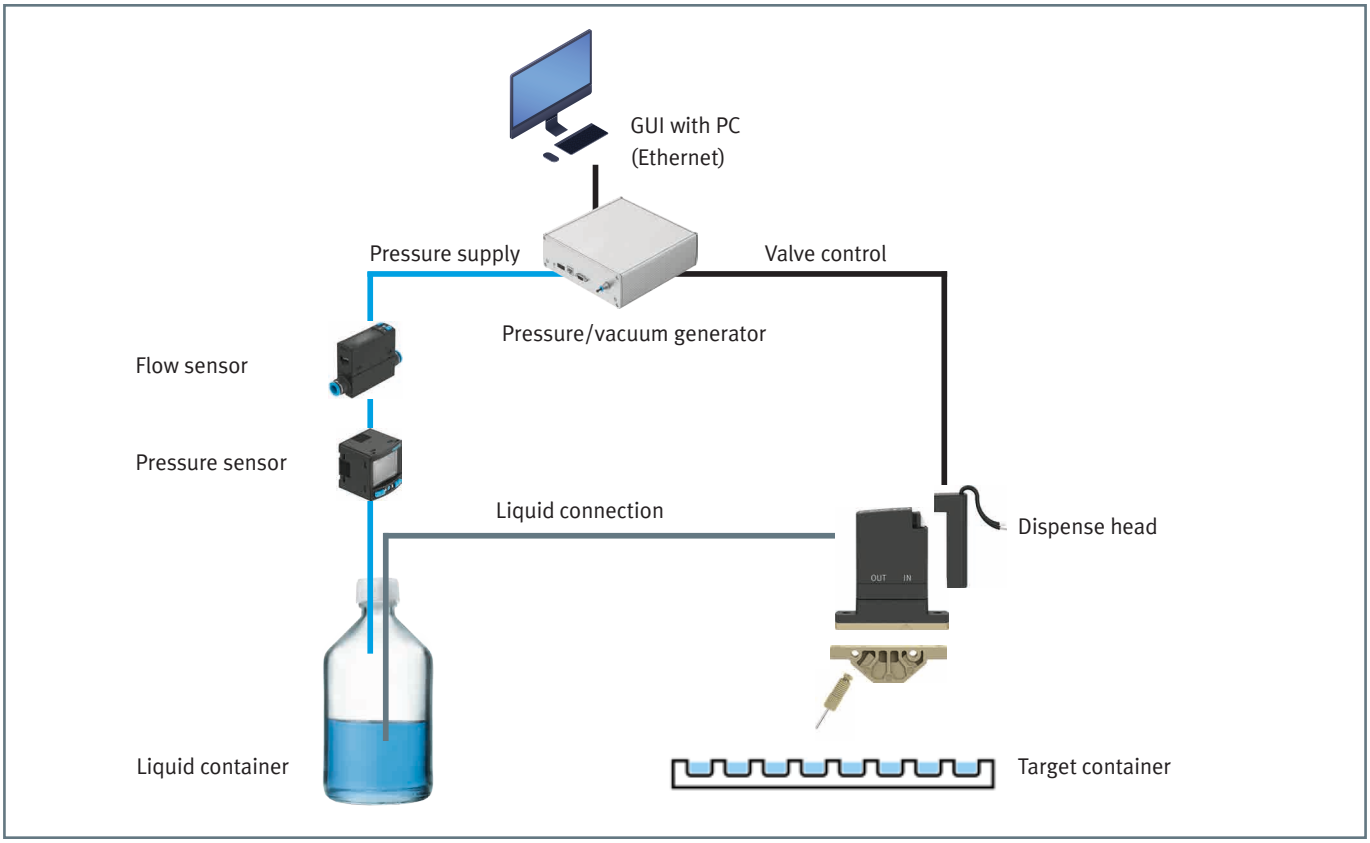
The assumption was that stress during dispensing does not necessarily lead immediately to cell death. Negative effects often only become apparent hours later as cell viability decreases.

Cell viability was analyzed for each variation using the CelltiterGlo® assay Promega according to the manufacturer's instructions. The prepared reagent was mixed with the cell suspension to be measured in a 96-well microwell plate at a ratio of 1:1 (100 µl each). As a reference, both reagent and cell suspension were pipetted manually. In the automated case, the cell suspension was dispensed using the Festo dispensing system and the reagent was then added. After an incubation period of 30 minutes and 24 hours at room temperature, the luminescence of the individual wells of the microwell plate was measured using a plate reader.

Incubation time:
30 minutes and 24 hours

Ratio 1:1
100 µl reagent to 100 µl cell suspension

Schematic setup of the dispensing system



Since the cells are kept in a closed container for a certain period of time and are constantly shaken before dispensing, cell viability and oxygen partial pressure were monitored over a period of 90 minutes.

Viability values between 96% and 100% were measured and an oxygen partial pressure above 95% was confirmed; this is an excellent result and thus a stable starting position for the actual dispensing tests.

Viability:
Average cell viability of over 90%

An average cell viability of over 90% was measured during the actual dispensing experiments, whereby no systematic dependencies between the varied process parameters (pressure, needle diameter, angle of inclination, distance) and the resulting viability were observed for an incubation time of 30 minutes.

Equivalent growth rates were also observed between automated dispensing and the reference when considering an incubation time of 24 hours. Only a direct comparison between needle diameters 0.3 and 0.6 mm shows that viability tends to decrease with very narrow cross-sections.

The results thus demonstrate that an automated dispensing system is fundamentally comparable with manual pipetting in terms of the resulting viability.

However, it should be noted that only the HEK293 cell line was examined. The extent to which the results can be transferred to other cell lines and how parameters such as pressure or needle diameter may need to be adjusted must be checked on a case-by-case basis.

Conclusion

Automation solutions for handling biological cells enable laboratories to increase their productivity, quality, and profitability. However, for these solutions to be used successfully, it is crucial that cell viability is impaired as little as possible. Festo offers a wide range of components for a modular and flexible dispensing system.

The pressure-controlled dispensing method allows parameters such as pressure and valve opening times to be adjusted to the cell suspension in order to minimize the impact on viability. Practical trials with a selected cell line have shown that the average viability of the cells was over 90%. It is therefore comparable to manually pipetted samples. The Festo dispensing system is a very good starting point for the gentle and precise handling of cells and offers all the benefits of automation. The modular design and the adjustability of the parameters make it possible to adapt the system to different cell lines and cell concentrations.

Publisher:
Festo SE & Co. KG
Ruiter Strasse 82
73734 Esslingen

Your contact person:
Ms. Paula Marin Canibano
paula.marincanibano@festo.com

Further information on automation solutions for LifeTech can be found online:
→ www.festo.com/lifetech