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Routine Challenges and Solutions in Cell Biology

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Executive Summary

Cell biology is a complex field in which basic research plays a prominent role, while at the same time eukaryotic cells serve as test systems. Work in a cell biology laboratory is characterized to a large extent by the routine tasks of cell culture. This White Paper will reflect upon the general requirements that comprise working with cells. These requirements refer to the condition of the cells, with a focus on the topics of contamination and viability, as well as on those processes in the cell biology laboratory that concern the occurrence of errors. The importance of homogeneous experimental conditions is also addressed. Approaches to solutions will be presented based on these considerations.

Introduction

Basic research concerns itself with the study of the composition of cells as well as with all cellular processes, including metabolism and cell division. In addition, cultured cells play a major role as test systems which serve as platforms for the study of the impact of substances on survival rates, metabolism and other processes within the cell. This area of application is particularly relevant to the field of pharmaceutical research, during the development of new drugs, but it is also applied in the cosmetics industry, where such experiments can replace animal testing of the tolerability of substances. Cell lines are further employed in biotechnological processes, for example, the large scale production of antibodies, vaccines and other therapeutic molecules.

Cells are cultured, manipulated and analyzed. Depending on the question, many analysis techniques overlap to a large extent with the methods of molecular biology and protein biochemistry - especially when the goal is to understand the function of genes.

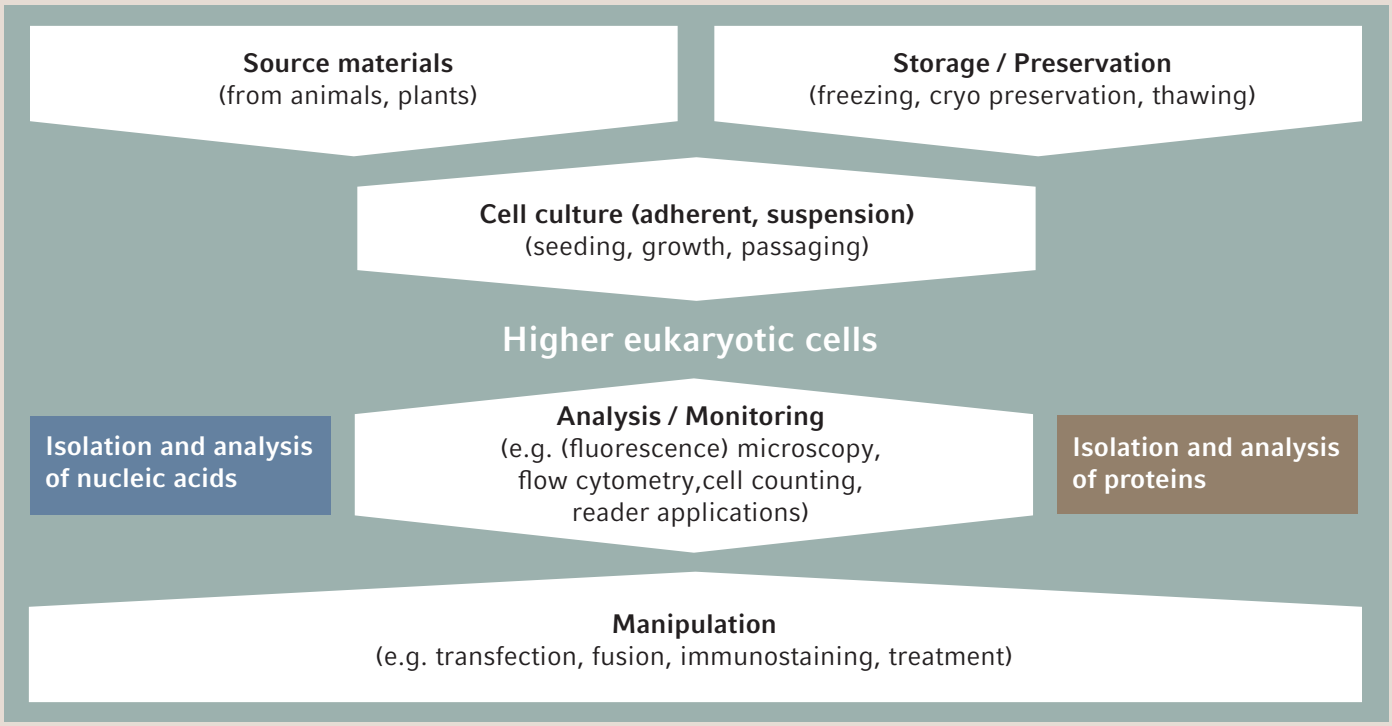


Figure 1: Standard techniques of the cell biology workflow

Figure 1 illustrates a simplified workflow of the techniques of cell biology, where all experiments in this area are based on cell culture. Cells are extracted from organisms in order to be able to establish *in vitro* cultures. *In vitro* cell culture requires the use of cell culture vessels, such as plates, flasks, dishes and, in certain cases, bioreactors. Alternatively, a cell line stock preserved in liquid nitrogen may constitute the starting material of a culture. Cultivation is typically carried out in three steps: cells are distributed into cell culture vessels, followed by the growth/proliferation phase, and once a certain cell density has been reached, cells are transferred to new vessels in a process that is generally referred to as passaging (figure 2). Continuous monitoring is essential in order to be able to control the condition of the cells and their growth. To this end, cells are routinely subjected to microscopy and counting techniques.

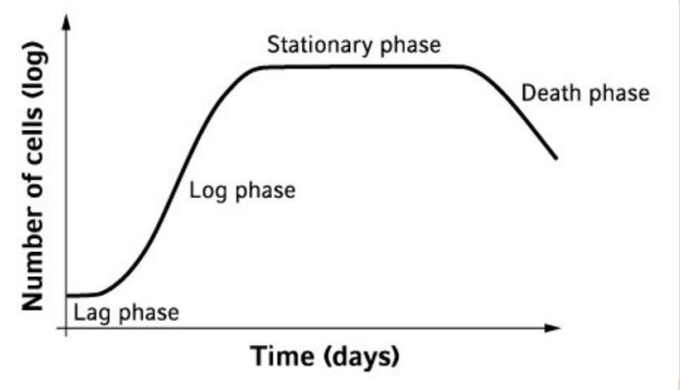


Figure 2: Cell growth curve
Lag phase: Cells recover from sub-cultivation, attach to the surface and start to spread.
Log phase: Cells grow exponentially and double at a characteristic rate defining the cell line's doubling time.
Plateau phase: The culture is confluent and cell growth slows or even stops.
Death phase: Cells start dying and detach from the surface.

The objective of the experiment determines the way in which the cultivated cells are treated or manipulated. Manipulation may encompass the introduction of molecules such as DNA via transfection, cell fusion, or the staining of their cellular structures. One important application includes cell-based assays, which utilize the cells as test systems. "Active agents" are added to the cells, and their impact on the cells is then analyzed via (fluorescence) microscopy or with the help of a reader. If nucleic acids or proteins are to be studied in subsequent analyses, molecular and biochemical methods may also be employed.

This White Paper centers on the general challenges of working in the field of cell biology, with a special concentration on cell culture. While not the primary focus of this paper, certain aspects of stem cells, microinjection and bioreactors will be addressed. Despite the fact that numerous different methods are routinely applied, cell work must satisfy certain requirements in order to guarantee reliable results. These requirements are described herein, and approaches to solutions will be presented.

Identification of challenges

It is the aim of all experiments to produce results that are accurate, precise and reproducible. Since they are based on live cells, these experiments will always be influenced by the state of the cells. The cells must be free from contamination, their identities must be secure, and their viability counts must be high. Laboratory processes must be carried out in a reproducible manner. In order to achieve and maintain these standards, work must be conducted under consistent conditions and errors must be avoided.

1. Condition of the cells

Cells are cultivated prior to their eventual use in assays and analyses. If these cells are contaminated, or if they are not in poor condition, the validity of the data will be questionable. It will then be necessary to repeat experiments, and further to the loss of potentially valuable sample material, time and money are wasted.

What types of contaminations exist, and from where do they originate? In most cases, cultured cells are contaminated with bacteria, yeast or mold that are most often introduced into the culture by the user. Since microorganisms find ideal growth conditions in cell culture, they tend to outgrow the cells within a very short period of time. Signs of contamination include a turbid culture medium as well as color changes due to altered pH values. Contamination with mycoplasma is more difficult to detect. While mycoplasma are considered bacteria, they do not possess a rigid cell wall and they are so small (figure 3) that they will pass through a 0.2 µm sterile filter.

Furthermore, mycoplasma cannot be detected using standard light microscopy. They attach to the membranes of cells, and the only way to detect them is through specialized assays. According to the DSMZ (German Collection of Microorganisms and Cell Cultures), 25% of all cell cultures worldwide are contaminated with mycoplasma [1]. Mycoplasma pose problems by consuming nutrients and by releasing toxins that impact the cells.

In order to lower the risk of contamination with bacteria, cell culture media are often supplemented with antibiotics. While this approach may be helpful in certain circumstances, it is by no means a universal solution. In addition to the danger of suppressing an existing contamination and thus fostering resistance, the cells themselves may experience damage, for example, through impairment of their metabolism [2, 3]. Furthermore, standard antibiotics are ineffective when it comes to mycoplasma. In order to reduce the need for antibiotics, or even do without them, cleanliness and sterile technique in cell culture are paramount.

Cross-contamination may lead to inter-mixing of cells; in extreme cases this situation will result in the complete replacement of the original cell line by another (figure 4). Falsely identified and cross-contaminated cell lines are published in the ICLAC database (International Cell Line Authentication Committee; version 8.0, December 2016). Based on these numbers, it is estimated that 15 – 20% of all human cell lines do not correspond to their stated source.
Most contaminations result from unclean or careless technique as well as from the users themselves. Additional sources include contaminated disposables, reagents or laboratory equipment, as well as dissemination via air, water or dust.

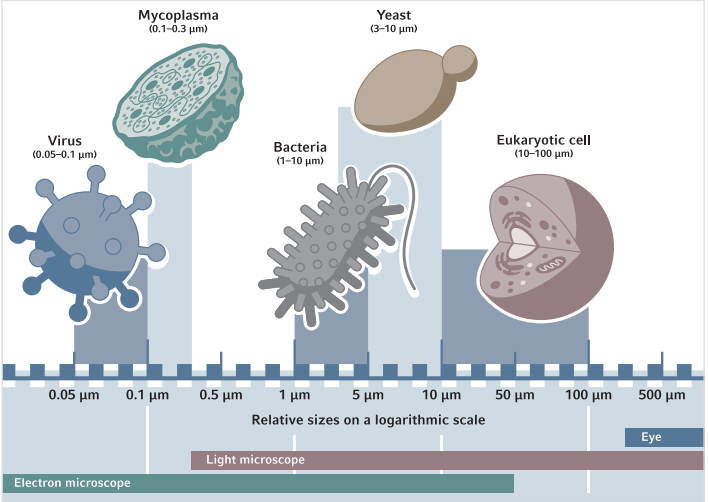


Figure 3: The different sizes of cells

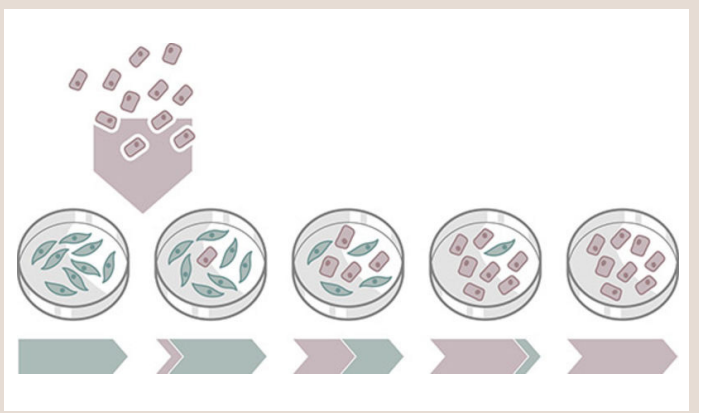


Figure 4: Displacement of the original cell line as a result of cross-contamination

While the condition of the cells, which is vital for the generation of valid results, is mirrored by their viability and their growth characteristics, it is also of interest to obtain information on cell-specific features. Such information is particularly important in the case of stem cells with their high differentiation potential. The challenge is to create, and maintain, physiological growth conditions for the cells – which include the temperature, pH value, nutrient composition and, if required, a growth surface suitable for the respective cell type. Defined cell culture media are standard for the cultivation of cells. In order to support cell growth and division as well as cell attachment, additional components such as hormones and growth factors are required, which are for the most part supplemented in the form of fetal calf serum. In addition, or as an alternative, it is customary to coat the surfaces of cell culture vessels in order to accommodate more challenging cell lines. The fact that these supplements and coating materials are frequently of biological origin, and that their components may therefore not be entirely defined, is problematic. Since substantial lot-dependent variation may be present, elaborate and costly testing is required.
Besides biological contaminations, chemical substances are capable of influencing the growth and the behavior of cells in culture. Scientific publications show that laboratory consumables made from plastic release substances which show effects in cell-based assays. The White Paper 26 [4] offers information on the topic as well as an overview of numerous scientific publications. Endotoxins, too, may impact cell culture [5]. Contaminations with this component of the cell wall of gram-negative bacteria can originate from consumables, from reagents or from DNA-constructs that were purified from bacteria. Problems may also arise from residual detergent following the cleaning of glassware and serological pipettes.

2. Laboratory processes

A large part of cell biology encompasses routine tasks in cell culture. Since the well-being of the cells is a priority, users often work under less than ergonomic conditions. Many steps must be carried out inside a biological safety cabinet and sterile technique must be upheld at all times, while under time constraints, and while processing large numbers of samples simultaneously. These pressures can lead to errors through fatigue or poor concentration, and as a consequence, samples may be mixed up, contaminated or dispensed incorrectly.

At the same time, even minute deviations from standard culture and processing procedures of the cells can impact the homogeneity of data and, as a result, the reproducibility of the experiments. Through altered parameters during the culture or the treatment of cells, the conclusion may change. Safeguarding a maximum of homogeneous growth and reaction conditions for all cells within an experiment, and ensuring adherence to these conditions during all subsequent experiments, independent of the user, thus presents an additional challenge. Many factors are influential: non-homogeneous growth may result from the fact that cells were seeded in inconsistent numbers, that air bubbles were introduced or that cells were not homogeneously dispersed within the medium. In addition to cell growth, the concentration of media components and test substances influence viability and cell metabolism. Differences can result from inaccurate pipetting or from evaporation, but also from the timing of the treatment. Variations in the surfaces of consumables may lead to less efficient adherence of adherent cells and, as a result, to compromised analyses.

Solutions & Benefits

1. Conditions of the cells

A) Prevention of contaminations

The highest priority when working with cells is consistent application of sterile working technique, which includes careful cleaning and regular tests in order to minimize the risk of contamination. Details are available in the standard literature on cell culture as well as in documents from Eppendorf [6, 7, 8, 9]. Contaminations can be avoided by utilizing consumables of appropriate purity grades, and the design of the product itself can contribute to the success of sterile technique through improved handling. The user must be careful to dispense all solutions in a contamination-free manner and ensure that cells are protected during incubation.

Pure consumables

Since consumables are in direct contact with the sample, their purity is essential. In addition to sterility, it is crucial that the cell culture consumables are free from endotoxins, as it is nearly impossible to remove these at a later time. The security that cell culture work demands is provided by consumables that are tested in a lot-specific manner and that are certified sterile as well as free from endotoxins. This is the case for Eppendorf consumables of the purity grades "Eppendorf Sterile" and Eppendorf Biopur® [10] as well as for all "Cell Handling" consumables (figure 5).

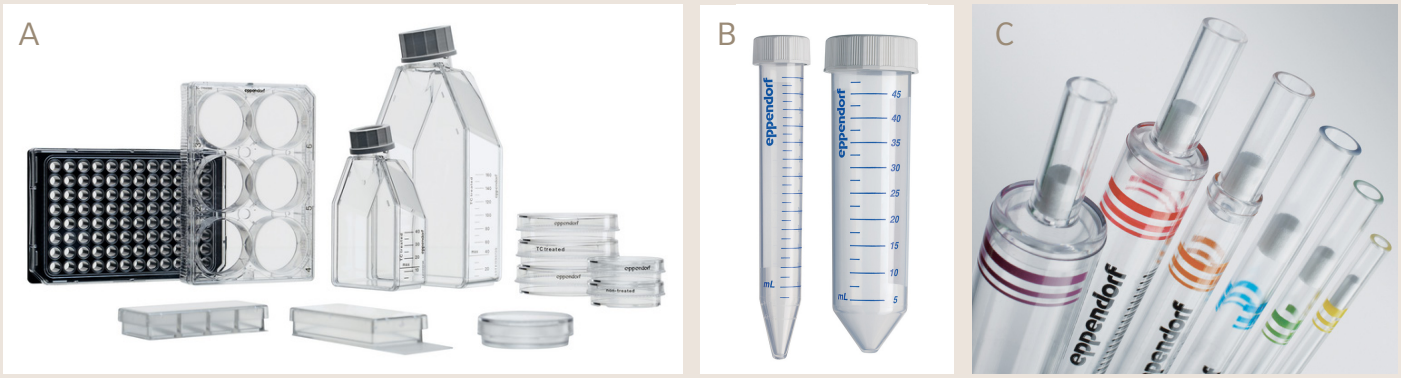


Figure 5: a) Eppendorf Cell Culture Consumables and Cell Imaging Consumables, **b)** Eppendorf Conical Tubes **c)** Eppendorf Serological Pipets

Functional design of consumables

When working with cells, it is imperative that correct and comprehensive sterile technique is followed. Defined product features will make these tasks easier and thus help avoid errors. The corrugated handling ring of the Eppendorf Cell Culture Dishes and the enlarged corrugated gripping area of the Eppendorf Cell Culture Plates simplify transport and prevent the lids from inadvertently being lifted (figure 6a+b).

The SplashProtect™ ring on the inside of the dish lid prevents contamination caused by splashes as well as by condensation (figure 6a). The wide opening of the Eppendorf Cell Culture Flasks (ConvexAccess™ geometry) facilitates access to the growth surface and thus makes cell treatment more comfortable and secure (figure 6c).

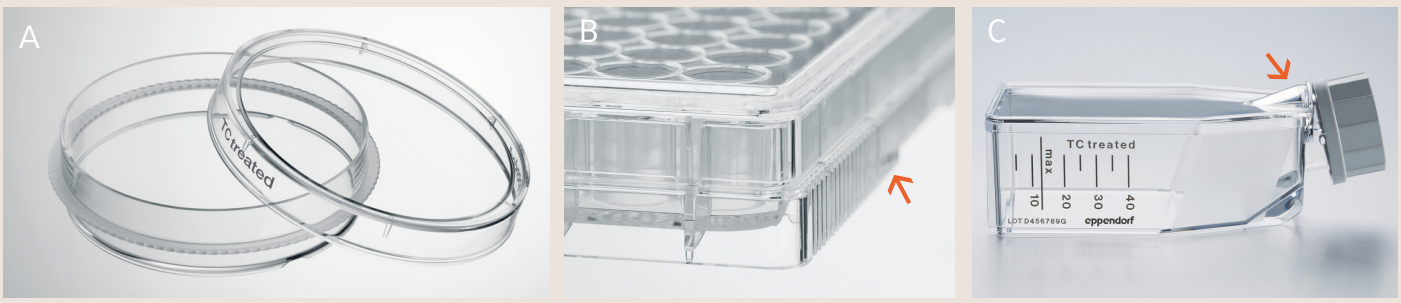


Figure: 6 a) Cell Culture Dish with handling ring and SplashProtect ring, **6 b)** Section of the Cell Culture Plate with gripping area (arrow), **6 c)** Cell Culture Flask with ConvexAccess neck geometry (arrow)

Contamination-free dispensing of solutions

Aerosols that form during pipetting can quickly lead to contamination of the inside of air cushion pipettes (figure 7a) and may thus be transferred to other samples. Filter tips with high retaining (e.g. ep Dualfilter T.I.P.S.®) effectively protect the inside of the pipette shaft. Direct displacement systems (figure 7b), such as the dispenser Multipette®/ Repeater® M4, are an excellent alternative, as their piston is integrated in the corresponding tips (Combitips advanced®) (figure 7c). As a result, liquids and aerosols are safely contained within the tip. 15 mL conical tubes are standard issue in cell culture (figure 8c). If pipette tips of "normal" length are used (figure 8b), the risk of the pipette cone touching the vessel wall and thus transmitting contaminations is high. This situation can be avoided by either using elongated tip variants (figure 8a) or by using shorter vessels such as, for example, the Eppendorf Tubes® 5.0 mL (figure 8d).

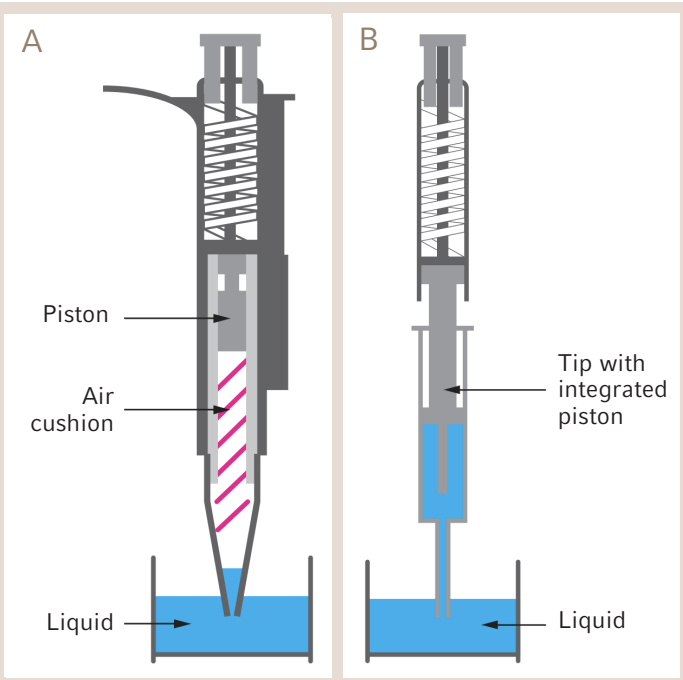


Figure 7: a) Pipette with air cushion principle,
b) Direct displacement system

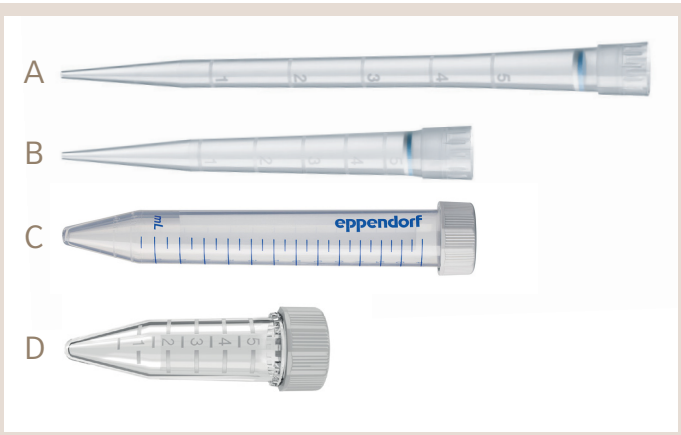


Figure 8: a) ep Dualfilter T.I.P.S. 5 mL L (elongated variant),
b) ep Dualfilter T.I.P.S. 5 mL (standard tip)
c) Eppendorf Conical Tube 15 mL,
d) Eppendorf Tube 5.0 mL

Secure incubation

Since CO_2 incubators provide ideal growth conditions for microorganisms, they harbor a substantial contamination risk. Only a consistent cleaning and decontamination routine will prevent microbial growth. It is therefore advantageous if a thorough cleaning routine can be carried out with as little effort and disruption as possible. In White Paper 30 [11], different strategies are compared with respect to incubator design: the inner chamber of the CO_2-incubators by Eppendorf is easily cleaned since it is manufactured from one piece, i.e. the inner chamber is entirely without seams and protruding screws. In addition, special temperature regulation technology enables the incubator to function without a fan, a potential source of airborne contamination [7, 12]. Additional features include automated high temperature disinfection, split inner doors as well as a copper chamber with antibacterial properties. Cell culture vessels must be ventilated while at the same time preventing contamination. Figure 9 shows how the high efficiency filters, which are integrated into the caps of the Eppendorf Cell Culture Flasks, provide more efficient protection from contamination than a conventional filter membrane [13].

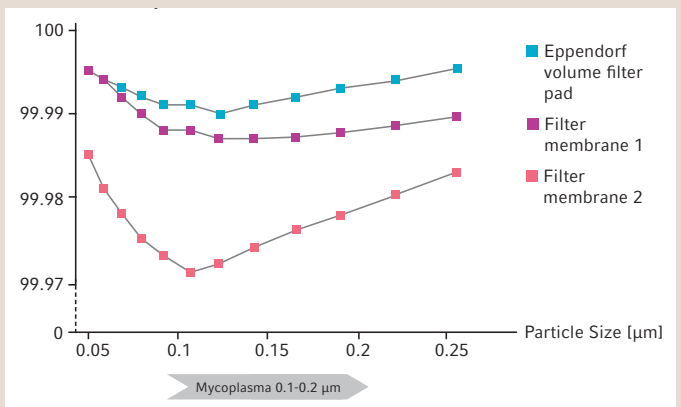


Fig. 9: The new air filter technology of the Eppendorf Filter Caps provides better protection from contamination than conventional filter membranes.

B) High cell viability

In order to achieve reliable cell growth, cells must be cultured under conditions which resemble their natural environment as closely as possible. In addition to the right temperature and a stable pH, the proper medium and a suitable growth surface are essential. Fluctuations are to be avoided and cells should be exposed to non-physiological conditions for as short a time as possible. Furthermore, damage by contact with cytotoxic substances is to be avoided.

Incubation under optimal conditions

It is one of the most important goals of cell culture to safeguard the stability of incubation conditions. Fluctuations translate into cellular stress, which may impact survival rates. One distinguishing feature of the Eppendorf CO_2 incubators is the fact that all six walls (including the door) can be heated directly, which results in superb temperature stability and homogeneity (figure 10). Each opening of the door, however, contributes to a change in the environment inside the chamber. It is therefore important that temperature, as well as gas composition, are restored quickly. The Eppendorf CellXpert® incubators, for example, are capable of restoring environmental conditions within 5 minutes. Split inner doors are also advantageous as they help maintain uniform conditions inside the chamber (figure 11).

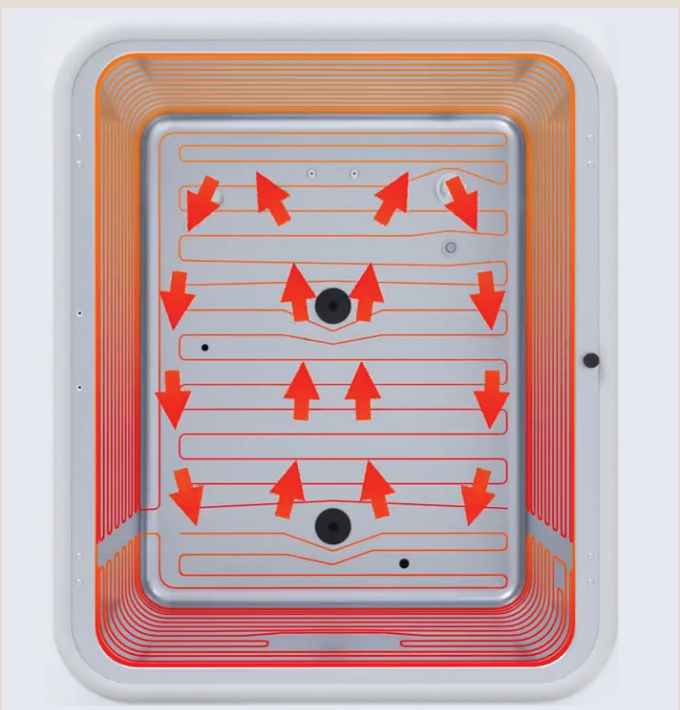


Figure 10: Direct heating of the chambers of the Eppendorf CO_2 incubators results in gentle convection circulation which ensures a reliable control of temperature and CO_2 content.

Minimizing time outside the incubator

It is only inside the incubator that conditions are geared towards optimizing cell growth and survival. In order to safeguard cell viability outside the incubator, processing steps should be carried out as quickly as possible – especially in the case of sensitive cells. The consumables used are capable of significantly influencing the speed and quality of analysis. Particularly in 96 well plates, the medium will form a meniscus which then complicates focusing during cell microscopy due to the disruption of the phase contrast. The same effect is generated by poor planarity. The precise design and treatment of the Eppendorf Cell Culture Consumables results in very even well-bottoms and the formation of a meniscus inside the well is minimized. The uniform illumination thus attained, free from disturbing shadows at the edges, facilitates focusing on the cells and thus accelerates the analysis process (figure 12). If cells need to spend more time outside of the incubator, filling the inter-well spaces of the plate will keep the temperature stable and prevent unwanted temperature shifts.

Figure 11: Split inner doors maintain the atmosphere inside the incubators.

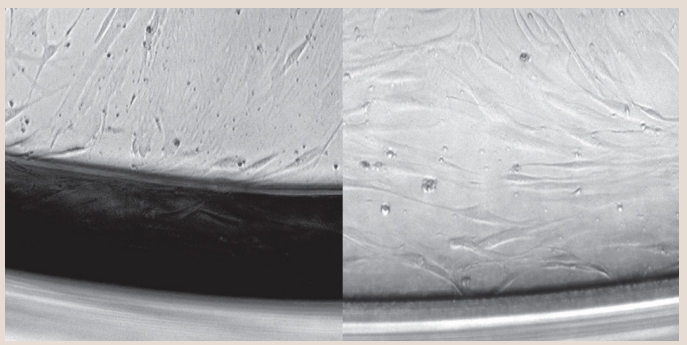
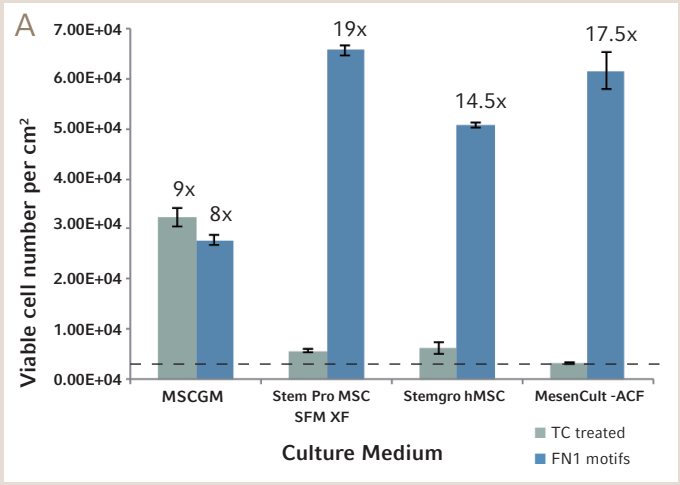


Figure 12: The well of an Eppendorf plate is fully illuminated under the microscope (right). An interfering shadow can be observed at the edge of the wells when visualizing a competitor plate under identical conditions (left).

Growth on defined surfaces

There is a trend towards cultivating cells under serum-free conditions, with supplements that are entirely synthetic [14]. Selected Eppendorf cell culture products are available which feature an entirely synthetic surface coating (CCCadvanced FN1 motifs) which contains neither animal nor human components. It was shown in Application Note 390 [15] that this novel surface is compatible with several commercially available media that are free from components stemming from other organisms (xeno-free). In this way, it was possible to establish an entirely defined culture system for human mesenchymal stem cells (hMSCs) as well as induced pluripotent stem cells (iPSCs). In addition to a high and stable growth rate the cells maintained their characteristic morphologies (figure 13), their characteristic marker expression profile and their ability to differentiate over long periods of time. It is thus possible to expand cells under controlled and reproducible conditions.



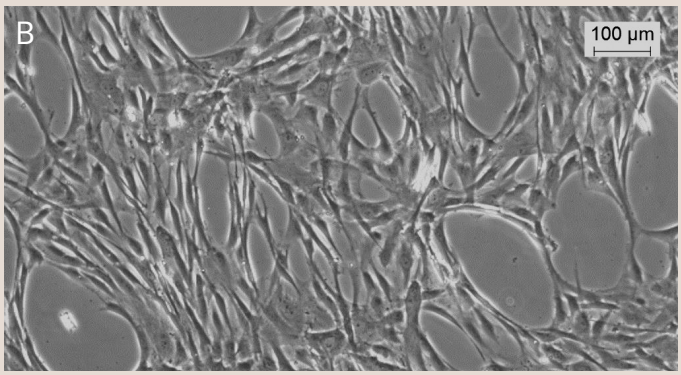


Figure 13: A) Proliferation of hMSCs following short-term expansion on Eppendorf CCCadvanced FN1 motifs using different commercially available cell culture media (the dotted line indicates the initial cell seeding density (3,500 cells/cm²); average fold inductions are noted above columns.) **B)** Characteristic morphology of hMSCs following short-term expansion on Eppendorf CCCadvanced FN1 motifs in Xeno-free medium (StemPro).

Avoiding leachables

Further to biological contaminations, chemical substances are also capable of compromising cell growth and distorting analyses. Eppendorf Consumables are exclusively manufactured from carefully selected raw materials and are entirely without plasticizers, biocides or mold release agents. All Eppendorf Cell Culture and Imaging Consumables, as well as the BioBLU® single-use bioreactors (figure 14), are tested for cytotoxicity. Application Note 308 [16] describes how the Eppendorf bioreactors are subjected to a leaching test [17]. Neither the growth nor the metabolic profile of CHO and Vero cells were found to be influenced. Figure 15 depicts the example of the survival rate of CHO cells.
Detailed information on the topic of leachables is available at www.eppendorf.com/consumables.

Figure 14: Eppendorf BioBLU 0.3c and 1c Single-Use Vessels for cell culture applications

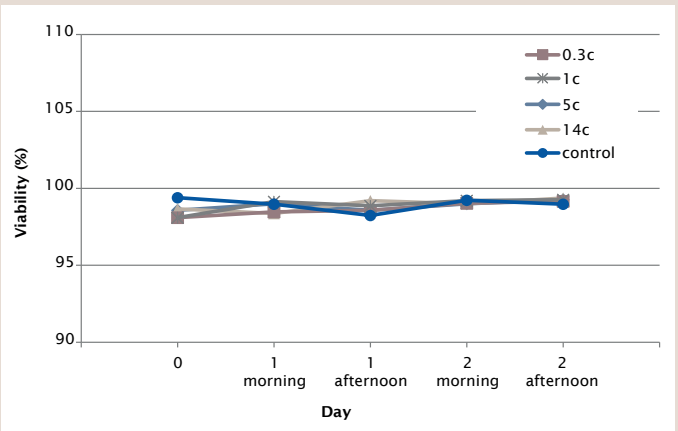


Figure 15: Viability of CHO cells which had been cultivated either in extraction media from BioBLU Single-Use Vessels or a shake flask (control)

2. Laboratory processes

A) Error avoidance

This chapter will cover the measures which may help prevent errors from occurring. In this respect, minimizing work-related stresses on the user is of prime importance. Automation and the use of ergonomic work equipment can contribute to the relief from routine tasks. Appropriate product features that offer visual support or provide intelligent software are also capable of contributing to a less cumbersome work routine. Additional information on Eppendorf products that contribute to improved ergonomic working conditions in the laboratory are available at www.eppendorf.com under "Physiocare".

Automation
If the user is relieved from routine tasks, particularly from dispensing liquids, potential sources of errors can thus be eliminated. The Eppendorf workstation ep*Motion*® (figure 16) is capable of automating medium-throughput cell-based assays, as these are mainly carried out in the 96 and 384 well formats and encompass many dispensing steps. Several Application Notes have described the successful completion of such assays using the ep*Motion* [18, 19, 20]. Using different programs, routine tasks such as cell seeding, compound dilution and addition, as well as addition of the assay reagent, can be accomplished with ease.

Figure 16: Eppendorf ep*Motion* with CleanCap option which offers a UV-lamp and a HEPA filter system for decontamination and clean air conditions.

Use of ergonomic working equipment
Different aspects and approaches to solutions on the topic of ergonomics in the laboratory are outlined in Userguide 46 [21], which describes the Eppendorf Physiocare Concept®. Since dispensing of liquids makes up a large part of daily laboratory work, it is especially important to reduce stressors in this particular area. A combination of appropriate laboratory equipment and the proper technique can minimize the risk of physical ailments suffered by laboratory staff. Eppendorf Liquid Handling Tools feature many ergonomic characteristics – among them the spring-loaded tip cone of all Eppendorf pipettes that ensures minimal attachment and ejection forces. This effect is shown in comparison with the predecessor model of the Eppendorf Research® (figure 17) [22]. The electronic pipetting aid Easypet® 3 (figure 18) was also designed with ergonomics in mind and therefore allows fatigue-free work [23].

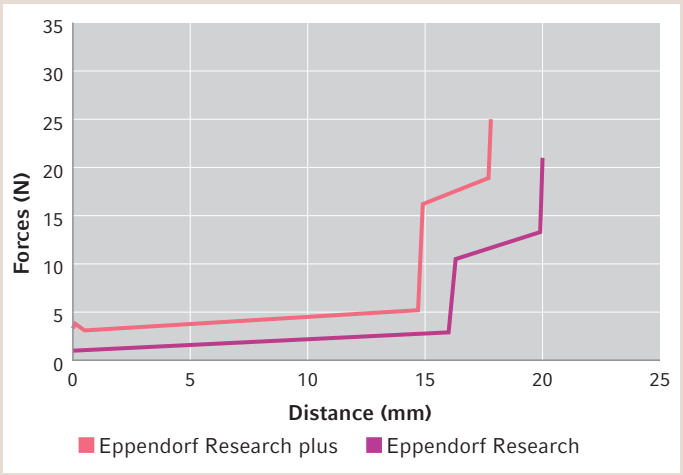


Figure 17: Pipetting forces (exclusive of tip ejection) of the Eppendorf Research plus in comparison with the Eppendorf Research.

Figure 18: Eppendorf Easypet 3

Visual support

Optical product features that offer helpful information contribute to the avoidance of errors as well as simplify readability. The Eppendorf Microplates, Deepwell Plates and Cell Culture Plates feature the OptiTrack® System – contrast-rich alphanumeric laser labeling of all positions (figure 19). In this way, wells are identified more easily and quickly and the risk of confusing samples is thus reduced. All Eppendorf Cell Culture Consumables are labeled according to their surface (TC treated or non-treated), so that the two variants are unique. Especially when many samples are processed in the plate format, careful and concentrated work is of the essence in order to fill each well with the correct solution. Electronic dispensing systems with step counters allow each action to be traceable. A manual dispenser with integrated step counter is also available in the form of the Eppendorf Multipette/Repeater M4 (figure 20).

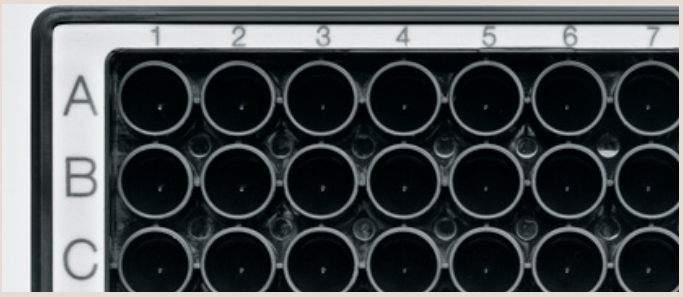


Figure 19: Laser labeling of the Eppendorf Microplate black

Intelligent software

Even the operation of laboratory equipment is not entirely free from error. The Eppendorf Xplorer® and the Eppendorf Multipette/Repeater E3x are electronic dispensing tools with intuitive user interfaces that facilitate easy handling. In addition, all Eppendorf Multipettes are further equipped with a sensor which recognizes the size of the attached Combitip advanced. Following selection of the number of dispensing steps, the display automatically shows the volume to be dispensed in each step (figure 20). Errors are avoided as no manual calculations are required.

Micromanipulation, which can be used for the injection of nucleic acids into cells, is a method that demands a skilled and experienced operator. The TransferMan® 4r (for example, for the injection of egg cells and early embryonic stages) (figure 21a) and the InjectMan® 4 (for example, for injection into adherent cells) are operated intuitively. In addition, they feature a selection of optimized user interfaces for various applications (figure 21b) as well as retrievable semi-automatic travel distances (e.g. position storage). These features simplify sophisticated work procedures and thus speeding up the workflow.

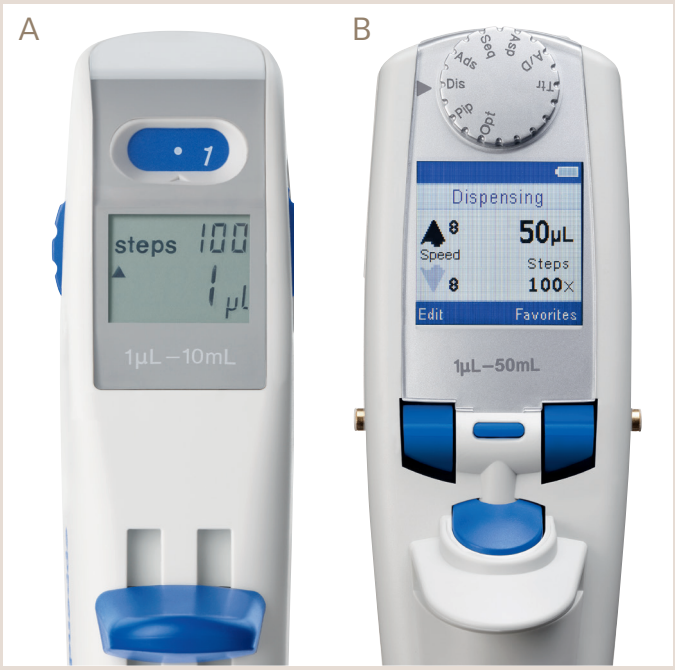


Figure 20: Display of the Eppendorf Multipette/Repeater M4 (a) and Multipette/Repeater E3x (b) showing a step counter as well as the dispensed volume.

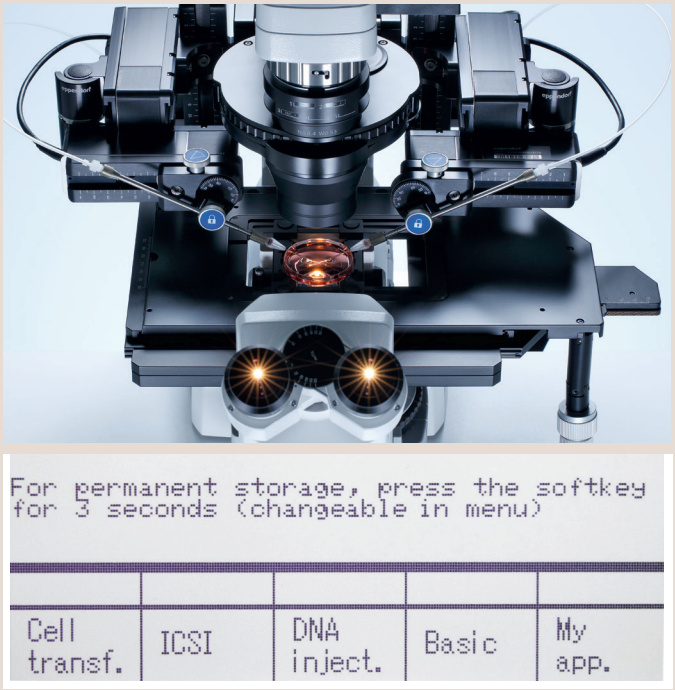


Figure 21: Eppendorf TransferMan 4r
a) Microinjection workstation
b) Display of the control panel with user interface for the selection of different pre-programmed applications

B) Homogeneity

In order to attain reliable conclusions from applications in the area of cell biology, it is not only stable growth and high cell viability that are essential; the experimental conditions themselves must also be consistent and reproducible. Conditions for homogeneous growth include uniform seeding and identical conditions for all cells. Within the scope of cell-based assays, the cells are treated with test substances. To this end, it is imperative that dispensing be performed accurately. Consumables of high and consistent quality are conducive to cell growth as well as to accurate analyses.

Uniform seeding

In order to ensure homogeneous density across all cells, cells must be seeded into the cell culture vessels in a homogeneous fashion. Since cells will sediment over time (figure 22), their numbers may vary considerably between different pipetted suspensions. In an effort to distribute the cells evenly and create a homogeneous suspension, cells are frequently resuspended. This approach, however, carries the risk of shear forces damaging the cells and air bubbles impeding their subsequent adherence to the plastic surface. Dispensers are very well suited to the task of dispensing cells into the culture vessels quickly and yet gently, while maintaining controlled conditions and avoiding the formation of air bubbles. In Application Note 350 [24], the use of different pipetting systems for routine tasks in cell culture is described in detail. It was shown that the cells were homogeneously distributed in the wells when the Multipette/Repeater M4 was used for cell seeding (figure 23).

Precise dispensing

Cell-based assays are mainly carried out in 96 and 384 well plates. The plates must be processed quickly and accurately in order to achieve reliable results. Deviations can result in altered cellular responses to test substances. When using the ep*Motion* 96 and the electronic 12-channel pipette Eppendorf Xplorer for a cell-based assay, significantly better reproducibility was achieved as compared to a manual 12-channel pipette [25]. The ep*Motion* 96 is a semi-automatic electronic pipette for simultaneous dispensing into 96 wells of a plate. It is capable of working considerably faster, which is critical in the case of time-sensitive assays (figure 24). Many tips on how to perform cell-based assays successfully are listed in White Paper 35 [26].

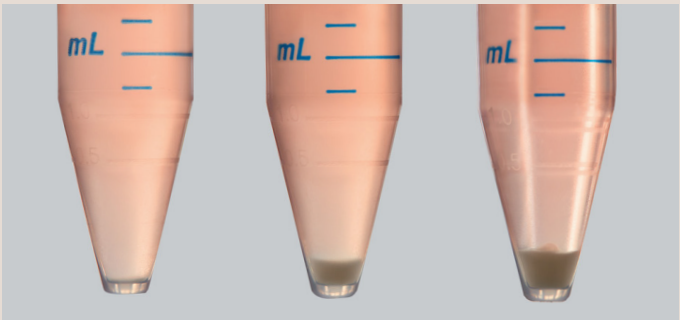


Figure 22: Sedimentation of cells in conical vessels within a few minutes without resuspending.

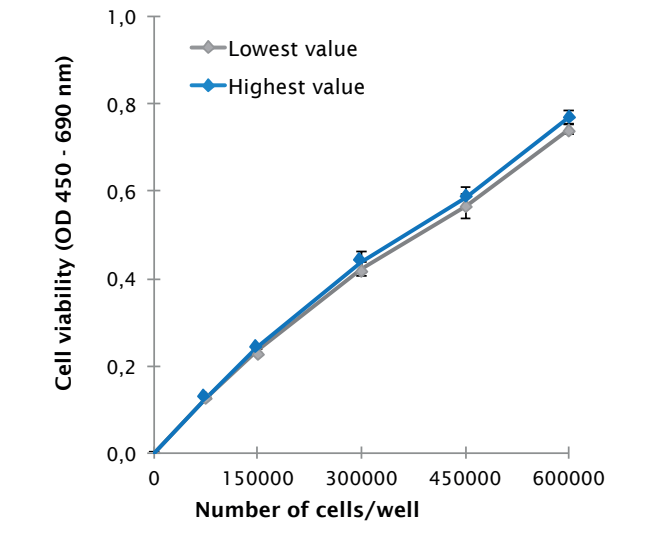


Figure 23: The number of viable cells was determined by a colorimetric assay following seeding six different amounts of cells into 24-well plates using the Multipette/Repeater M4. Four replicates were performed for each condition, of which the highest and lowest values are displayed.

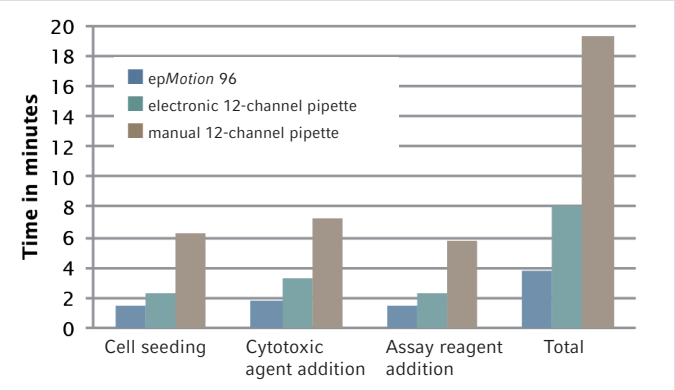


Figure 24: Hands-on time required for processing one assay plate using different instruments.

Homogeneous growth conditions

The selection of the cell culture plate, too, will influence cell growth. Especially 96 well plates, owing to the low working volume, are prone to high evaporation rates in the edge wells. As a result, media components as well as metabolic products and, if present, test substances accumulate and may thus affect cell behavior. Due to the edge effect, the outer wells of plates are frequently not used, which translates to a loss of 38% of the total capacity of the plate.

Figure 25 shows that filling of the outer moat of the Eppendorf Cell Culture Plates (figure 26) reduces evaporation, particularly in the peripheral wells of the plate [27]. More uniform conditions bring about more homogeneous cellular reactions across the entire plate, as shown here by means of the example of cell proliferation in different types of plates (figure 27) [28].

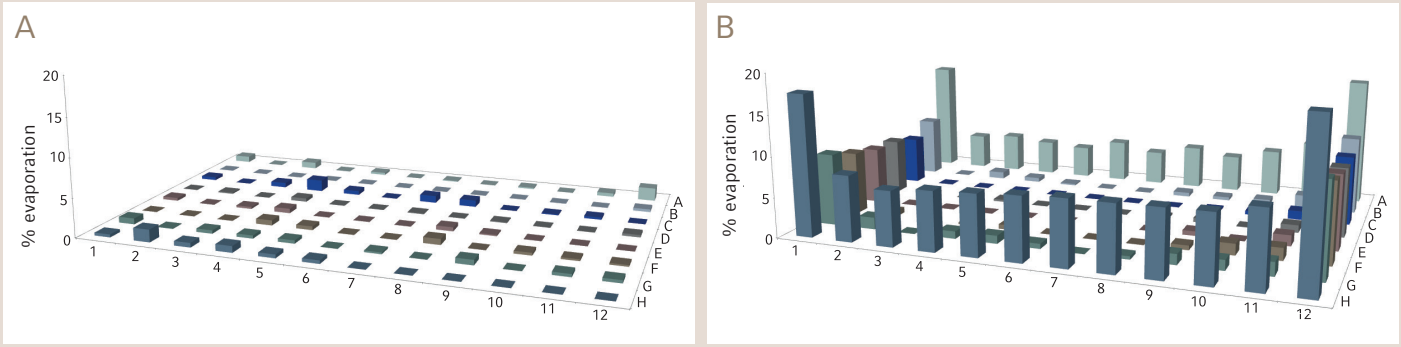


Figure 25: Comparison of evaporation rates in 96-well cell culture plates following 5 days of incubation:
a) Eppendorf Cell Culture Plate, **b)** competitor plate

Figure 26: Eppendorf Cell Culture Plate with filled outer moat

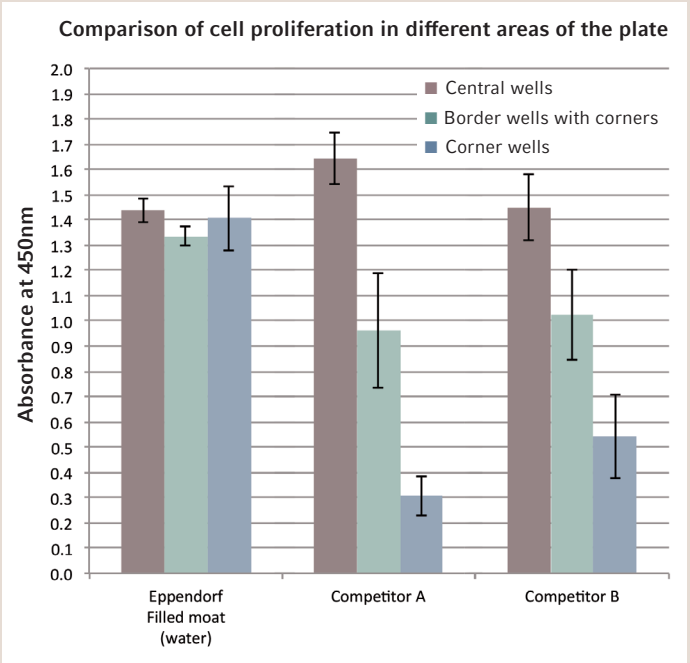


Figure 27: Comparison of cell proliferation in different areas of cell culture plates after 7 days of incubation (mean of three independent replicates (n=3)).

Accurate analyses

High quality consumables distinguish themselves through uniformity within the product as well as through minimal deviations between manufacturing lots. The TC treated surface of the Eppendorf Cell Culture and Imaging Consumables is very homogeneous. The product performance of each batch is tested and certified with respect to cell adhesion and cell growth.

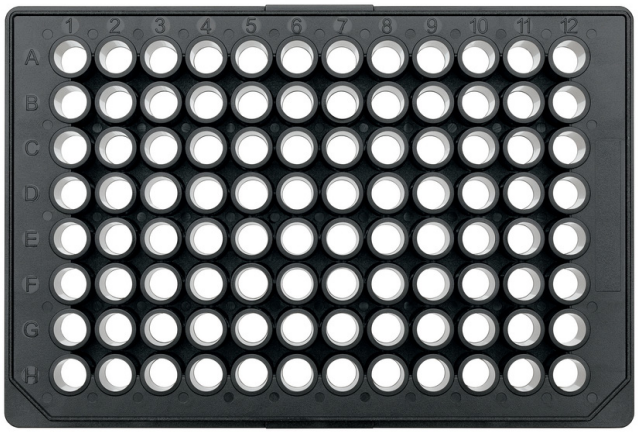


Figure 28: Eppendorf Cell Imaging Plate 96 wells

The Cell Imaging Plates (figure 28) are available with either glass bottoms or foil bottoms. They are very robust, and they show excellent light transmission, while the black plate body suppresses cross interference of signals between the wells. In comparison with plate bottoms made from conventional polystyrene, the material of the Eppendorf Cell Imaging Plates exhibits considerably lower autofluorescence (figure 29), which results in a good signal-to-noise ratio.

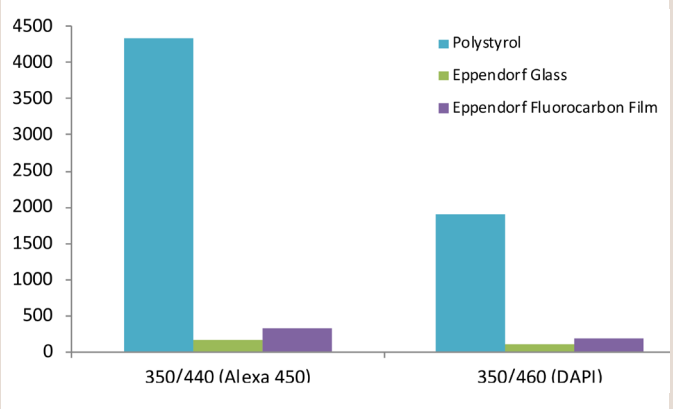


Figure 29: Autofluorescence of plates, with bottoms made from different materials.

Promotion

Numerous information on cell biological products and applications is available on the Eppendorf website (www.eppendorf.com) under the following tabs and areas:

> Products: In this section, individual Eppendorf products are described in detail.
> Applications: Here is an overview of the utilization of Eppendorf products in selected workflows and application areas.
> Service & Support: This is the place where, as a source of knowledge, the FAQ collection and the "Knowledge Base", which contains all available documents such as instruction manuals, brochures and Application Notes, are stored. In addition, videos are available in the Media Center. The Eppendorf Training Center lists classroom training courses as well as planned and recorded webinars.

The start page also leads to the section "Eppendorf Handling Solutions" (https://handling-solutions.eppendorf.com/), which comprises a compilation of basic facts and knowledge on the topic of cells.

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Since 1945, the Eppendorf brand has been synonymous with customer-oriented processes and innovative products, such as laboratory devices and consumables for liquid handling, cell handling and sample handling. Today, Eppendorf and its more than 3,000 employees serve as experts and advisors, using their unique knowledge and experience to support laboratories and research institutions around the world. The foundation of the company's expertise is its focus on its customers. Eppendorf's exchange of ideas with its customers results in comprehensive solutions that in turn become industry standards. Eppendorf will continue on this path in the future, true to the standard set by the company's founders: that of sustainably improving people's living conditions.

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