BIO NEWS
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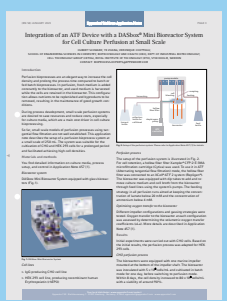
eppendorf


# mAb Scale-Up: Efficient and Cost-Effective

> Eppendorf Tubes® BioBased for more sustainability
> Centrifuge 5427 R: now with hydrocarbon cooling
> How to brush up your pipetting knowledge

**Application Notes**

Isolation and enrichment of Golgi bodies from rice seedlings · Loop-mediated isothermal amplification (LAMP) of human apolipoprotein L1 gene · etc.


Integration of an ATF Device with a DASbox® Mini Bioreactor System for Cell Culture Perfusion at Small Scale

# Thank You

for making time for our new issue of Eppendorf BioNews. With our wide-ranging selection of topics, we would like to offer you helpful suggestions and ideas – in the familiar way that you have come to expect from BioNews over the past 30 years. With information on workflows, products, and initiatives, as well as on the subject of sustainability at Eppendorf.

Our leading article reveals what is key when it comes to the development of a robust, efficient, and cost-effective research and development process for mAbs (p. 4–5). On page 7, you will learn more about Eppendorf twin.tec® Trace PCR Plates, our latest and most advanced PCR plates. "Academic Research Takes Center Stage" is our motto on page 9, where we present our current initiative "Your Work Matters".

Once again, we address the topic of sustainability: we are introducing the new Centrifuge 5427 R with hydrocarbon cooling (p. 8) as well as our Eppendorf Tubes® BioBased (p. 6), which are manufactured from a starting material that originates from renewable, reused raw materials. Eppendorf is pursuing four concrete sustainability goals: climate change, natural resources, social compliance, and social well-being. More on this topic on page 10 (incl. download option).

"It was really fun to solve the crossword and problem with my lab – great team-building opportunity!", writes a reader from Canada. You, too, are invited to take the opportunity to tackle our puzzle as a team – once again, the first prize is a valuable electronic Eppendorf Xplorer® plus 8-channel pipette.

Enjoy!

Your Eppendorf BioNews team

**PS:** Would you like to share your thoughts with us about BioNews? Do you have ideas or requests? If so, please send us an e-mail to bionews@eppendorf.de. We look forward to your feedback!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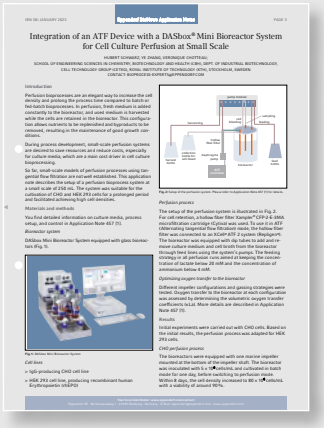
## Eppendorf BioNews Application Notes


Integration of an ATF Device with a DASbox® Mini Bioreactor System for Cell Culture Perfusion at Small Scale

DAVID SOLBACH, EPPENDORF SE BIOPROCESS CENTER, JUELICH, GERMANY

# Efficient and Cost-Effective Monoclonal Antibody Scale-Up

**Propelled by the COVID-19 pandemic, the pressure increases to develop more products for treating unexpected viral diseases. Products must be available in a timely manner while ensuring that manufacturing is cost-effective and under safe conditions. Monoclonal antibodies (mAb) may hold great promise here as an addition to vaccines and anti-viral drugs. However, satisfying market needs with competitive products entails several hurdles and risks. How can manufacturers tackle the challenges?**


When it comes to a fast time to market or staying ahead in the market, time is money. The early stages of cell line development and scale-up are key to the overall success of the process. Establishing a highly robust, efficient, and cost-effective mAb workflow requires an early process characterization. Reliable and stable processes must be developed, featured by reproducible cell growth, consistent high viability of cells, and maximized yield. Highest efficiency with reduced manual handling steps is paramount. Single-use technologies and process-intensification help addressing these goals. Employing continuous manufacturing strategies results in higher yields and can help to reduce the costs, compared to classical batch and fed-batch approaches. A reproducible transfer from small scale to larger volumes is the prerequisite for a fast time to market and for mitigating risks with respect to supply chain and market access.

## Process development – maximizing yields and process stability

Challenge and goal is always to maximize the yield, which depends on the viability and productivity of the cells. Each cell line and even each clone performs differently.

To ensure stable cell growth profiles and antibody yields from small- and bench-scale to production-scale requires a strategy based on a detailed process understanding and optimized bioreactor conditions.

Media optimization, designing a better expression system, or studying the metabolic pathways can increase productivity. Precise equipment such as pumps, sensors, and gas control is the prerequisite to ensure stable and consistent process runs. Proper instrumentation allows to monitor metabolic pathways, assessing productivity consequences of, for example, carbon source depletions and optimize conditions in the bioreactor.

Analytical equipment with sensor integration in parallel systems is superior for process insights. Different settings, parameters and cell lines can be tested at early stage at the same time. The parallel control of multiple bioreactors and simultaneous monitoring of various process parameter speeds up process characterization and increases time and cost efficiency. Software-supported process control with sensor-driven feedback loops enables automation, reduces manual handling steps and the risk of human errors, thereby increasing safety, reproducibility, and efficiency.

### Scale-up – maintaining cell growth profiles and antibody yields

One of the major mAb production challenges is cell culture scale-up. Studying the reactor conditions help cells to grow effectively and to produce a proper amount of product.

Essential for efficient mixing and mass transfer throughout the different bioreactor sizes is an optimal vessel geometry based on the ratio of impeller diameter to vessel diameter. Popular scale-up strategies enable a reproducible transfer from small scale to larger volumes. However, calculations are complex and require expert knowledge.

Software-guided calculations of important process parameters save time, considerably ease process setups, and mitigate the risk of failures. Combined with new open-concept controllers, similar cell growth profiles can be produced in a variety of bioreactors, regardless of supplier, from bench to production scale.

### Device management – preventing downtimes and failures

Cutting corners in selecting equipment could have a big and potentially negative impact on the process.

The worst-case scenario leads to batch failures that cause supply shortages of critical medication.

A system should support industry standards, give insights about its status, and send reminder if service is required. Industries with a large base of installed equipment will particularly benefit from that. Digital sensors that provide information about their lifetime allow to replace a sensor that is close to end of life. This can lead to significant cost savings as risks of process failures due to defect sensors are avoided.

Instrumentation suppliers should provide any data on the suitability of the equipment for a specific process. Being familiar with problems manufacturers face, they can provide expert support based on a common understanding.

### Conclusion

Development of a robust, efficient, and cost-effective monoclonal antibody research and development process is accompanied by many hurdles and risks. With the right and reliable equipment, process development and optimization at bench, pilot and production scale will lead to success.

More information at

**News**

## GIFs – now also from Eppendorf

GIFs – who doesn't know them, these short animated image sequences that depict emotional, iconic, or funny moments. GIFs can be used to express your mood or spice up comments, whether in video chats or in popular social media apps.

The Eppendorf Online team has been creative and productive, creating a range of different animated and real GIFs – with the special help of volunteers from among our colleagues, who put in a good effort to shoot the animations. You can see they had fun doing it!

The use of the moving images is very easy. Simply type in the keyword #Eppendorf in the GIF library of your app or visit https://giphy.com/eppendorf/ and off you go.


Have fun using our GIFs!

BRIGITTE KLOSE, EPPENDORF SE

# 2nd Generation Feedstock – 1st Class Consumables

**Imagine that your laboratory tube could be manufactured from renewable raw materials, as well as from waste and residues generated by the vegetable oil industry. Would you have faith in the quality of the product? Would you depend on the purity and reliability of the tube and entrust it with your valuable samples – in the same way that you have always trusted your tube made from fossil raw materials? If this thought has piqued your interest, continue reading.**


Plastic vessels manufactured from fossil raw materials have long replaced glass containers in the laboratory and have become irreplaceable. With respect to sustainability, this constitutes a considerable challenge, for laboratories and manufacturers alike. We have therefore made it our goal to produce high-quality plastic products for the laboratory in a more sustainable fashion and employ renewable resources of the second generation in the process.

### From used vegetable oil to top-quality laboratory consumables

When selecting alternative, more sustainable materials, our plastic experts decided in favor of a biobased polymer which is produced from renewable, reused raw materials such as, for example, used cooking oil. The Eppendorf Tubes® BioBased manufactured from these raw materials are a new generation of vessels that allow us to help you achieve your sustainability goals.

### Biobased polymer – what exactly is it?

With the biobased polymer chosen by Eppendorf, fossil raw materials are saved through their replacement with sustainable raw materials made from waste materials and residues of used cooking oil (also known as "second generation biofuels"). These raw materials are traceable to the first oil collection facility. The production of this polymer is certified with the sustainability certificate "ISCC PLUS". With an ISCC PLUS certification, companies ensure that the entire supply chain, from cultivation to the final product, has been audited.

ISCC supports the transition to a circular economy and bioeconomy, bringing with it many advantages such as the traceability of the entire supply chain, authentication of the identity of raw materials, clear information verified by third parties, and the strengthening of consumer trust through an independent certification system.

### No compromise

Eppendorf Tubes BioBased are equal to fossil-based Eppendorf Tubes with respect to both quality and performance. All quality guarantees issued with our product-specific, as well as lot-specific certificates are equally valid for Eppendorf Tubes BioBased. The switch to Eppendorf Tubes BioBased does not pose a risk to your samples or your experimental data.

More information:
www.eppendorf.com/BioBased

TIM SCHOMMARTZ, EPPENDORF SE

# twin.tec "Trace": The Next Generation

**Eppendorf twin.tec® PCR Plates are an established plate format in the laboratory. Their thin-walled polypropylene wells and robust polycarbonate frame ensure optimal heat transfer and high dimensional stability. These materials confer extreme durability on the twin.tec PCR Plates during incubation and centrifugation as well as in automated processes.**

### NEW: "Trace" – for even better traceability

Eppendorf twin.tec Trace PCR Plates are our latest and most advanced PCR plates. An exciting new design and innovative features guarantee outstanding performance.

The laser-engraved lot number and expiry date on each single plate improve traceability in your laboratory processes and thus make these new plates an excellent choice for all regulated workflows. The innovative optical guiding grid, together with the proven OptiTrack® matrix, enables quick orientation within the plate which, in turn, simplifies manual and semi-automated pipetting and dispensing. Raised well rims allow an effective sealing and minimize the risk of cross-contamination.

### … and even more color

While the traditional classic twin.tec PCR Plates are already colorful, the new colors of the twin.tec Trace PCR Plates – crystal blue and fuchsia – provide even more variety in the laboratory, plus they improve readability and labeling contrast.

Their outstanding reproducibility, as well as other specifications, are equivalent to those of the traditional twin.tec PCR Plates. This makes the twin.tec Trace PCR Plates a perfect choice for high-throughput applications as well as automated workflows and standardized processes.

twin.tec Trace PCR Plates are also available with barcode; they are batch-tested and certified free from DNA, DNase, RNase, and PCR inhibitors (PCR clean).

More information at
https://www.eppendorf.com/plates

**News**

## Differentiation through Uniqueness

Do you work with DNA, proteins, or light-sensitive samples? Get the best out of your applications now with the new Eppendorf Conical Tubes 25 mL DNA LoBind® and Protein LoBind or Eppendorf Conical Tubes 25 mL Amber. Available either with screw cap or with our patented* SnapTec® cap.

Tubes with DNA LoBind or Protein LoBind surface are ideal for use in applications where concentrations tend to be small and sample recovery is vital for assay results.


Handling light-sensitive samples in non-transparent long tube formats is a frequent source of contamination. The new 25 mL amber colored tubes combine effective protection from high-energy light in the low wavelength range with a high degree of transparency.

www.eppendorf.com/25mL

*US Patent 8,540,948

NICOLE SEELIGMÜLLER, EPPENDORF SE

# Centrifuge 5427 R: Now with Hydrocarbon Cooling

**Global warming and its consequences are among the most pressing challenges of our time. Fluorinated hydrocarbons, which had been used until recently in cooling systems such as air conditioning units, but also in laboratory centrifuges and freezers, can, upon their release, contribute to global warming due to their chemical structure. Therefore, in order to protect our planet for future generations, it is important to switch to more environmentally friendly "green" coolants – hydrocarbons – even in the laboratory.**

Natural coolants have a low global warming potential (GWP) of <3 – similar to $CO_2$. In contrast, conventional coolants, including the coolant R134a, which is used in refrigerated centrifuges, exhibit a GWP of 1,430 and thus, when released, exert a substantially higher effect on global warming than natural coolants.

In order to contribute to the protection of the environment within the scope of the laboratory, Eppendorf is now offering the new Centrifuge 5427 R with hydrocarbon refrigeration. It constitutes the first centrifuge in our portfolio which operates with a natural refrigerant. For the protection of your samples – and our planet.

## Global Warming Potential (GWP)

Perhaps you are asking yourself right now: what exactly is GWP? GWP is the index for the (relative) contribution of a defined mass of a chemical compound to the greenhouse effect, i.e., the average effect on the warming of Earth's atmosphere compared to an identical mass of $CO_2$ (in 100 years). This means that 1 kg of R134a with a GWP of 1,430 will, upon release, contribute 1,430 times more to global warming than 1 kg $CO_2$.

The terms "natural", or "green", refrigerants, respectively, comprise hydrocarbons with the substantial advantage of a low GWP. The refrigerants most commonly used in the laboratory environment are propane (R290) – for instance, in the Centrifuge 5427 R – and ethane (R170).

## Low GWP – high performance

The new Centrifuge 5427 R convinces not only with its reliable propane-based cooling system. With its compact footprint and the dual row rotor FA-45-48-11 for up to 48 x 1.5/2 mL tubes, it is the optimal solution, especially for laboratories with high sample throughput. Areas in which many users share the instrument will also benefit from its large selection of rotors: a total of nine fixed-angle and swing-out rotors cover a broad bandwidth of applications within the fields of molecular biology and cell biology.

# Isolation and Enrichment of Golgi Bodies from Rice Seedlings Using Density Gradient Ultracentrifugation

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## Introduction

The Golgi apparatus of eukaryotic cells was first described more than 120 years ago by Camillo Golgi. Advances in (electron) microscopy revealed the complex structure while further biochemical analysis enlighted various functions of this organelle within the cell [1].

In cells of higher organisms, the Golgi apparatus is responsible for the synthesis of complex polysaccharides and the processing and distribution of proteins to other organelles as part of the secretory pathway [2].

One example of such a protein is α-amylase, a glycosidase responsible for the hydrolysis of starch molecules within plants. It was shown that α-amylase is synthesized at the endoplasmic reticulum (ER) ribosomes, glycosylated within the ER-lumen, and then transported into the Golgi apparatus for oligosaccharide modification [3].

However, as the Golgi apparatus forms a complex structure with other membrane systems like the endoplasmic reticulum (ER) [1], it is particularly difficult to isolate distinct parts of this organelle. Indeed, fractions of Golgi membranes are often contaminated with parts of other connected membrane systems like the vacuole [1]. Density gradient centrifugation is one of the most established techniques used for the enrichment of specific membranes [1].

In this Application Note, we describe a technique using a sequence of differential pelleting and density gradient centrifugation to obtain fractions of Golgi apparatus membranes from rice seedlings. The applied technique allows to gain extracts of high purity and quality for further downstream analysis like mass spectrometry.

## Materials and methods

*Materials used*

Centrifuge CP80NX (Eppendorf) with the swing-bucket rotors P32ST for 40 mL PET tubes and P40ST for 13 mL PET tubes.

*First step*

Microsome purification process by using rotors P32ST (40 mL PET tube) and P40ST (13 mL PET tube).

1. Centrifuge the purified rice extract at 15,000 × *g* for 30 min at 4 °C in 40 mL PET tube in a swing-bucket rotor and discard the pellet.
2. Load the 11 mL supernatant on the top of 1 mL of 15% sucrose solution over the 1 mL of the 50% sucrose cushion in the 13 mL PET tube.
3. Centrifuge at 100,000 × *g* for 3 h at 4 °C and subsequently collect the microsome fraction trapped on the cushion of 50% sucrose solution.

*Second step*

Golgi purification process from microsome fraction by using rotor P40ST (13 mL PET tube).

1. Adjust the collected fraction to 42% sucrose density with 60% sucrose buffer using a refractometer. On top of this solution load 1–2 mL of another discontinuous sucrose density gradient consisting of 1 mL 26%, 30%, 34%, and 38% sucrose layer each. Fill carefully up with water to 13 mL.
2. Centrifuge at 100,000 × *g* for 3 h at 4 °C and subsequently collect the Golgi fraction (1) floating as boundary phase between 34% and 38% sucrose layer briefly.
3. Adjust the collected Golgi fraction to 42% sucrose density again, and then apply 1–2 mL to the second discontinuous sucrose gradient consisting of 1 mL 26%, 30%, 34%, and 38% sucrose layer each. Fill carefully up to 13 mL.
4. Centrifuge at 100,000 × *g* for 3 h at 4 °C and collect the Golgi fraction (2) floating as boundary phase between 34% and 38% sucrose layer.

All sucrose concentrations based on w/w.

## Results and discussion

The use of discontinuous density gradient centrifugation is a standard method for isolating or enriching subcellular components. In most cases, differences in the sedimentation coefficient or specific densities are used to obtain a separation. The characteristics regarding these parameters in organelle isolation applications are mainly defined by the composition of the respective membranes. A clear separation is often a challenge, especially for Golgi bodies closely connected with other membrane systems [1].

Hence, highly purified Golgi membranes are essential for the analysis and investigation of e.g. the Golgi proteome [2] or specific proteins within the organelle. Here, we describe an effective method using two different swing-bucket rotors in combination with successive discontinuous sucrose density gradient centrifugation steps to obtain high-quality isolates of Golgi bodies.

After removing the cell debris in the first centrifugation step, the supernatant is loaded onto a first discontinuous sucrose gradient (Fig. 1). Between the 15% and 50% sucrose phases, a fraction of microsomes is accumulated. This fraction is used for further purification by two steps of floating discontinuous sucrose gradients where the Golgi bodies accumulate between the 34% and 38% sucrose phase of the gradient (Fig. 2).

It was shown by Asakura *et al.* [4] that the purity of the Golgi body fraction was improved significantly after the second floating step.

# Isolation and Enrichment of Golgi Bodies from Rice Seedlings Using Density Gradient Ultracentrifugation

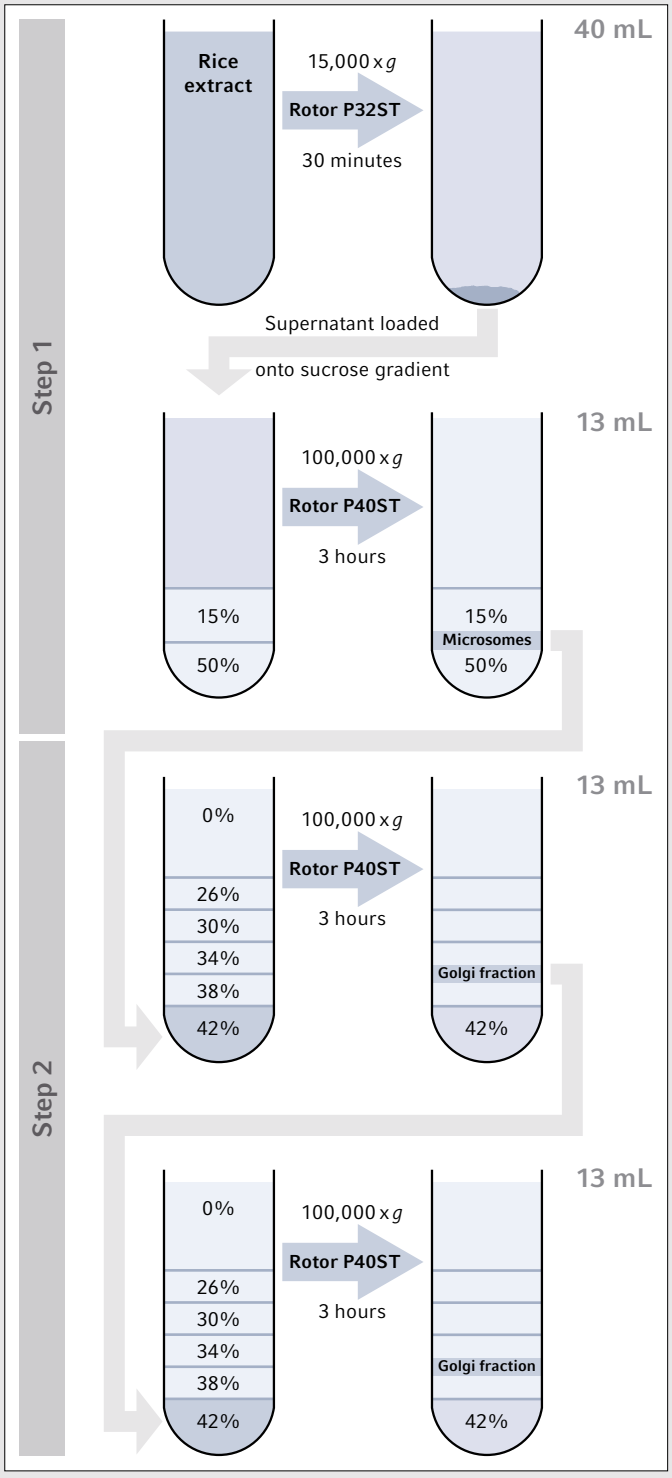


**Fig. 1:** Isolation of Golgi bodies by a sequence of discontinuous sucrose gradient centrifugation steps

The quality of the Golgi fraction can be checked by the presence of several marker enzymes (e.g. UGPase [Uridindiphosphate-Glucose-Pyrophosphorylase: Cytosol], RbcL [Ribulosebisphosphate carboxylase large chain: plastid] by immunoblot analysis [2].

## Conclusion

The combination of the two rotors P32ST and P40ST is ideal for the isolation of Golgi bodies. It allows the shift from higher (40 mL) to lower volumes (13 mL) with high performance. The special long and narrow shape of the 13 mL PET tubes allows a longer floating distance, which increases the purity of the Golgi fraction. Besides, the top-loading of the rotor inserts eases the delicate handling of sucrose gradients and minimizes the risk of unintended mixing.

Download of full Application Note 444:

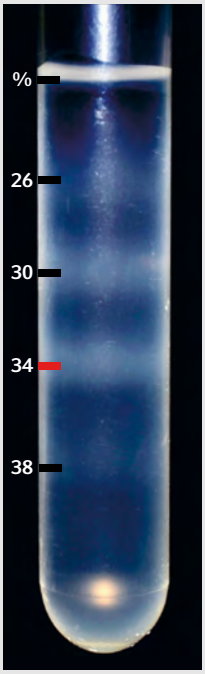


**Fig. 2:** Sub-fraction of the rice Golgi apparatus after multiple steps of discontinuous sucrose density gradient centrifugation. Golgi fraction between 34% and 38% sucrose solution indicated in red

## Literature

[1] Parsons H.T. *et al.* (2012): The Current State of the Golgi Proteomes, *Proteomic Applications in Biology*, Dr. Joshua Heazlewood (Ed.).

[2] Oikawa K. *et al.* (2018), *Methods in Molecular Biology*, Chapter 6, 91-105.

[3] Kitajima A. *et al.* (2009). *The Plant Cell*, Vol. 21: 2844-2858.

[4] Asakura T. *et al.* (2006): *Plant Biotechnology* 23, 475–485.

# Integration of an ATF Device with a DASbox® Mini Bioreactor System for Cell Culture Perfusion at Small Scale

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## Introduction

Perfusion bioprocesses are an elegant way to increase the cell density and prolong the process time compared to batch or fed-batch bioprocesses. In perfusion, fresh medium is added constantly to the bioreactor, and used medium is harvested while the cells are retained in the bioreactor. This configuration allows nutrients to be replenished and byproducts to be removed, resulting in the maintenance of good growth conditions.

During process development, small-scale perfusion systems are desired to save resources and reduce costs, especially for culture media, which are a main cost driver in cell culture bioprocessing.

So far, small-scale models of perfusion processes using tangential flow filtration are not well established. This Application Note describes the setup of a perfusion bioprocess system at a small scale of 250 mL. The system was suitable for the cultivation of CHO and HEK 293 cells for a prolonged period and facilitated achieving high cell densities.

## Materials and methods

You find detailed information on culture media, process setup, and control in Application Note 457 [1].

*Bioreactor system*

DASbox Mini Bioreactor System equipped with glass bioreactors (Fig. 1).

**Fig. 1:** DASbox Mini Bioreactor System

*Cell lines*

> IgG-producing CHO cell line

> HEK 293 cell line, producing recombinant human Erythropoietin (rhEPO)

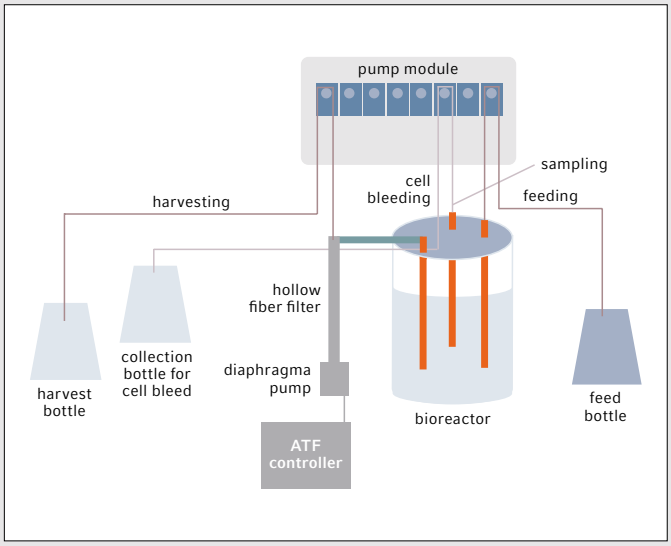


**Fig. 2:** Setup of the perfusion system. Please refer to Application Note 457 [1] for details

*Perfusion process*

The setup of the perfusion system is illustrated in Fig. 2. For cell retention, a hollow fiber filter Xampler® CFP-2-E-3MA microfiltration cartridge (Cytiva) was used. To use it in ATF (Alternating tangential flow filtration) mode, the hollow fiber filter was connected to an XCell® ATF 2 system (Repligen®). The bioreactor was equipped with dip tubes to add and remove culture medium and cell broth from the bioreactor through feed lines using the system's pumps. The feeding strategy in all perfusion runs aimed at keeping the concentration of lactate below 20 mM and the concentration of ammonium below 4 mM.

*Optimizing oxygen transfer to the bioreactor*

Different impeller configurations and gassing strategies were tested. Oxygen transfer to the bioreactor at each configuration was assessed by determining the volumetric oxygen transfer coefficients (κLa). More details are described in Application Note 457 [1].

## Results

Initial experiments were carried out with CHO cells. Based on the initial results, the perfusion process was adapted for HEK 293 cells.

*CHO perfusion process*

The bioreactors were equipped with one marine impeller mounted at the bottom of the impeller shaft. The bioreactor was inoculated with $5 \times 10^6$ cells/mL and cultivated in batch mode for one day, before switching to perfusion mode. Within 8 days, the cell density increased to $80 \times 10^6$ cells/mL with a viability of around 90 %.

# Integration of an ATF Device with a DASbox® Mini Bioreactor System for Cell Culture Perfusion at Small Scale

Throughout the process, the IgG sieving coefficient (ratio of product concentration in the harvest to product concentration in the bioreactor) was between 91 % and 99 %.

When the cell density exceeded $80 \times 10^6$ cells the process control deteriorated. The DO demand of the high-density culture could not be fulfilled, causing the DO to drop below the setpoint of 40 %. A high stirring speed was used and a high gas flow rate of up to 0.07 VVM was sparged into the bioreactor in the attempt to control the DO at setpoint, which caused excessive foaming. The viability stayed high, indicating that the CHO cells tolerated the suboptimal process conditions.

*HEK 293 perfusion process*

In a first step, the experimental setup described above for CHO cell cultivation was tested for the cultivation of HEK 293 cells producing rhEPO. In contrast to CHO cells, these cells did not tolerate the unfavorable process conditions, which occurred at high cell densities and which are described above. Therefore, the oxygen transfer to the bioreactor needed to be optimized. Based on preliminary experiments [1], a configuration with one Rushton impeller placed at the headspace/liquid interface and one marine impeller placed at the bottom of the impeller shaft was chosen.

The bioreactors were inoculated with $1 \times 10^6$ cells per mL. Around day 8 the cell density reached $20 \times 10^6$ cells/mL with viability of approximately 90 %. The cell density was maintained at this value for 14 days by applying cell bleeding. Subsequently, the cell density was stepwise increased by interrupting the bleed function up to $80 \times 10^6$ cells/mL with a viability around 95 %. The process was kept stable at these values for one week (Fig. 3). Therefore, by optimizing oxygen transfer, a cell density comparable to the previous runs with CHO cells was reached.

The concentration of rhEPO was quantified at various time points and the volumetric productivity was calculated. It increased linearly with cell density, from approximately 129 mg/L/day at cell densities of 15 to $25 \times 10^6$ cells/mL to approximately 392 mg/L/day at densities of 70 to $80 \times 10^6$ cells/mL.

## Conclusion

Cell densities of up to $80 \times 10^6$ cells/mL were maintained at a viability > 90 %, showing that high cell densities could be achieved at small scale, even though the bioreactor was slightly undersized for the ATF system used.

The described perfusion setup was suitable for process optimization at small scale. This Application Note describes only a part of the process optimization, namely oxygen transfer to the bioreactor. When optimizing a perfusion process, additional parameters need to be considered. Several experiments were conducted to analyze, how temperature, feed composition, and perfusion rate influenced process productivity and cell metabolism [2]. These findings demonstrate the potential of the presented small-scale perfusion bioprocess system for screening process parameters at small scale in parallel in a time- and cost-saving manner.

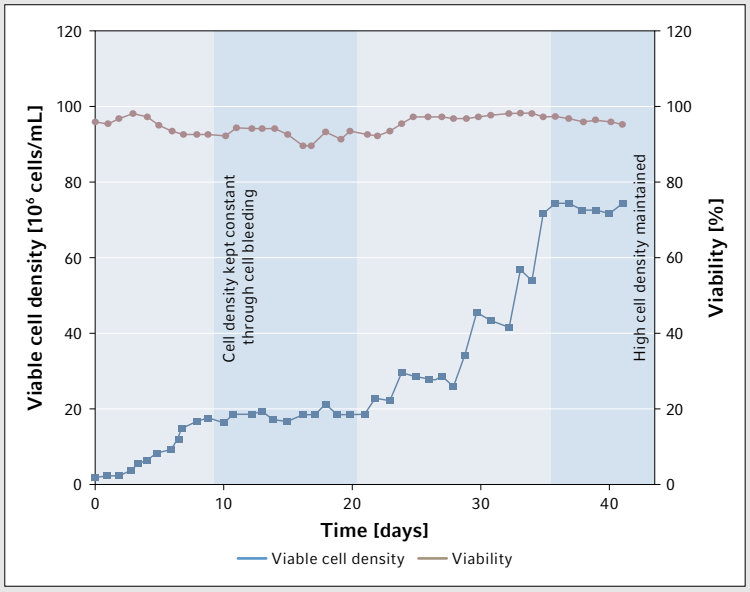


**Fig. 3:** HEK 293 cell bioprocess in perfusion mode

## Literature

[1] Schwarz, *et al.* Integration of an ATF Device with a DASbox Mini Bioreactor System for Cell Culture Perfusion at Small Scale. *Eppendorf Application Note 457.* 2022. Download at

[2] Schwarz, *et al.* Small-scale bioreactor supports high density HEK293 cell perfusion culture for the production of recombinant Erythropoietin. *J. Biotechnol.* 309 44–52. 2020. https://www.sciencedirect.com/science/article/abs/pii/S0168165619309538

# Optimized Isolation of Mononuclear Cells via Software Controlled Acceleration and Braking Ramp in the Centrifuge 5702 Family

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## Introduction

Fast sample processing is important in every laboratory. Since centrifuges are used in almost every workflow in molecular and cell biology as well as in microbiology, they can contribute significantly to the optimization of work processes. The continuous adaptation of centrifuges to the applicational needs of the customer is essential. This includes improvements of software as well as of hardware features. The implementation of a "Timer" function or the recently launched Vacutainer® buckets for the Centrifuge 5702 family are two examples. This Application Note is about the development of a "Soft Function" for the Centrifuge 5702 family and shows how cooperation between academic research and an industrial development team allowed the adaptation of a device to meet the requirements of a standard application.

With the models 5702/R/RH Eppendorf offers reliable centrifuges for applications that do not require g-forces above 3,000 x *g*. Originally, these centrifuges were developed both with a short acceleration and braking time to enable time savings in routine workflows. However, these high braking speeds (deceleration) can be a disadvantage in sensitive applications like density gradient centrifugation. To enable successful performance of these applications in the Centrifuge 5702 models as well, special attention was paid to the development of an additional acceleration and deceleration ramp that is gentle and yet fast.

Many research applications rely on viable and functionally intact cell populations. The isolation of human mononuclear cells (MNCs) via density gradient centrifugation is a standard method to achieve this. We therefore chose this protocol for our purpose. This separation technique is based on the density properties of Ficoll® [1]. While the high-density erythrocytes pass the Ficoll phase and form a sediment, the lymphocytes, thrombocytes, and monocytes are collected at the plasma gradient phase and thus can be enriched and used directly for subsequent experiments. During development, different software variants for the Centrifuge 5702 were investigated regarding their suitability for this application.

## Materials and methods

The new software variants were tested in a Centrifuge 5702. Since the bigger benchtop Centrifuge 5810 with swing-bucket rotor A-4-44 and corresponding adapters for 50 mL conical tubes is known to achieve good results in this application, it was chosen as the reference model. Centrifuge 5810 has ten acceleration and braking ramps, whereby level 0 (lowest acceleration and brake) was selected for this study.

Isolation of human mononuclear cells was carried out in 50 mL conical tubes using a Biocoll separating solution. To evaluate the quality of the separation in this experiment, a visual assessment first determined whether a defined interphase with clearly delineated phase transitions was visible. Subsequently, the number of cells per phase was counted to verify the separation process in terms of quantity.

1. 10 mL Biocoll separating solution (contains Ficoll® 400, GE Healthcare) at room temperature is pipetted into a conical 50 mL tube.
2. Human blood is diluted 1:2 with HBSS (Hanks balanced salt solution, Invitrogen®).
3. The Ficoll phase is covered with a layer of 35 mL diluted blood. It is essential that blood and Biocoll are not mixed.
4. Centrifugation for 30 min at 440 x *g* with the Soft function switched on and off (Centrifuge 5702, rotor A-4-38, adapter 5702 734.004 for 50 mL conical tubes).
5. Optical analysis of the resulting gradients using a digital camera.
6. Then the HBSS/thrombocyte/blood plasma supernatant is carefully pipetted off to approx. 1 cm above the MNC ring.
7. The MNCs are carefully transferred to a fresh 50 mL vessel using a 10 mL pipette. All the steps are performed on ice and no more than 20 mL per vessel is processed.
8. HBSS is used to top up the MNC level to 50 mL. The mixture is shaken thoroughly and centrifuged with the brake switched on (10 min, 440 x *g*).
9. When the supernatant has been removed by pipette, the pellet is resuspended in 1 mL HBSS and step 8 (centrifugation) is repeated.
10. Finally, the supernatant is removed by pipette and the cells are absorbed by 5 mL culture medium. The number of cells is counted (Neubauer counting chamber) and their size is determined. For this purpose, 50 µL of the cell suspension is mixed with 10 mL counting fluid (Isoton II, Beckmann Coulter) and analyzed in a Coulter Channelyzer 256 (Beckmann Coulter).

## Results and discussion

The results were initially documented with regard to quality by the visual assessment of the gradients and finally verified with regard to quantity by counting the number of cells. Care was taken that each original sample could be used to form at least four gradients: two in the Centrifuge 5702 model and two in the reference Centrifuge 5810.

First, the Centrifuge 5702 was operated with the brake switched on for all the necessary centrifugation steps. Besides careful layering of the whole blood and medium mixture on to the Ficoll (see material and methods), isolation of the mononuclear cells was not possible as no MNC ring could be detected. In the next step, a Soft function was implemented that enabled slightly prolonged acceleration and unbraked stopping of the rotor. The visual inspection proved that a detectable gradient was formed (Fig. 1a).

A comparison with the results obtained with the Centrifuge 5810 revealed that the gradient formed in the comparison

# Optimized Isolation of Mononuclear Cells via Software Controlled Acceleration and Braking Ramp in the Centrifuge 5702 Family

centrifuge was significantly more distinct (photo not shown). This optical impression is confirmed by the cell yield summarized in Table 1.

A significant improvement in the centrifugation results was achieved by using Centrifuge 5702 with a further developed software program (variant II). This variant enables smooth and slow acceleration as well as smooth, electronically controlled braking. A comparison of the two software variants clearly reveals that the ring of mononuclear cells is considerably easier to locate in the centrifuge with the improved variant II (Fig. 1b) and the resulting cell yield is higher (Table 1) than in the first variant I.

In a third step, the software was optimized to enable the braking time to be as short as possible without compromising the successful phase separation. With this variant III, both the formation of the gradient (Fig. 1c) and the yield of cells is comparable to variant II. Additionally, it enables considerably shorter braking times (approx. 60 s in comparison to approx. 180 s for variant II). In this way, it was possible to develop and implement an optimized software that enables to obtain a maximum yield of clean, well-separated, and viable MNCs and, additionally, reduce the time required for rotor deceleration.

Download of full Application Note 464

## Literature

[1] Böyum, A. Isolation of mononuclear cells and granulocytes from human blood. *Scand.J.Lab.Clin.Invest* 1968; 21:77-89.

| **Centrifuge** | **Centrifuge 5702** Software I | **Centrifuge 5702** Software I | **Centrifuge 5702** Software II | **Centrifuge 5810** (Reference) |
|---|---|---|---|---|
| | Program for Ficoll gradients | | | |
| rpm; rcf | 1,700 rpm; 440 x *g* | 1,700 rpm; 440 x *g* | 1,700 rpm; 440 x *g* | 1,600 rpm; 440 x *g* |
| Time (total) | 30 min | 30 min | 30 min | 30 min |
| Brake, acceleration | Max. | Soft function | Soft function | Min. (ramp 0/0) |
| | Washing program | | | |
| rpm; rcf | 1,700 rpm; 440 x *g* | 1,700 rpm; 440 x *g* | 1,700 rpm; 440 x *g* | 1,600 rpm; 440 x *g* |
| Time (total) | 10 min | 10 min | 10 min | 10 min |
| Brake, acceleration | Max. | Max. | Max. | Max. (ramp 9/9) |
| Amount MNCs | Not detectable | 152 million | 168 million | 172 million |

**Table 1:** Description and results of experiments for determining the optimal Soft function


**Fig. 1a-c:** Comparison of the gradient formation in Centrifuge 5702 with the Soft function switched on, software variant I (a); software variant II (b); software variant III (c)

# Loop-Mediated Isothermal Amplification (LAMP) of Human Apolipoprotein L1 Gene

NINA EGELER, STEFFEN RIETHMÜLLER, ARORA PHANG,
EPPENDORF SE, HAMBURG, GERMANY

## Abstract

The recent COVID-19 pandemic showed a silver lining in the molecular biology world by spurring on improvements in many methods especially in the detection of nucleic acids. "Loop-mediated isothermal amplification" (LAMP) has become a popular detection method due to its operation simplicity and rapidity. Using the WarmStart® Colorimetric LAMP 2X Master Mix (New England Biolabs®), the human apolipoprotein L1 gene (*ApoL1*) was detected in just 30 min with the Eppendorf ThermoMixer® C and the Eppendorf ThermoTop®. Additionally, even very low template DNA amounts of 0.1 ng were detectable by simply extending the incubation time to 45 min.

## Introduction

The amplification of nucleic acids is a widespread tool for the detection of various organisms and pathogens. Besides commonly used PCR-based methods, isothermal amplification offers an alternative for the detection of nucleic acids. Recently, the interest in isothermal amplification grew rapidly because of the COVID-19 pandemic. Some isothermal amplification methods are now already in use as PoC-NAT-tests (Point of care nucleic acid amplification tests) for the Coronavirus.

One of these methods is loop-mediated isothermal amplification (LAMP). LAMP was developed in 2000 by Notomi *et al.* [1]. During the reaction, a dumbbell-shaped DNA loop is created through primer annealing and the amplification is performed by polymerases with strand-displacement activity. This eliminates the need for an additional denaturation step and leads to continuous amplification of DNA under isothermal conditions. By adding reverse transcriptase to the assay, LAMP can also be used for the detection of RNA.

Successful LAMP reactions can be instantly observed through turbidimetry or colorimetric dyes without the necessity of an additional time-consuming step like gel electrophoresis. In this Application Note, we show the detection of the human *ApoL1* gene with two different polymerases and the following examination of its detection sensitivity.

## Materials and methods

Parts of *ApoL1* were amplified in 0.1 mL Eppendorf PCR Tube Strips from human genomic DNA (Promega®). See full Application Note 454 for detailed information*.

*Assessment of test sensitivity*

To observe how quickly lower template DNA amounts can be detected, the template DNA was diluted, and the color of the colorimetric dye was examined at different timepoints. The final samples contained the WarmStart Colorimetric LAMP 2X Master Mix with 0.001 ng, 0.01 ng, 0.1 ng, 1 ng, 10 ng, 50 ng, or 100 ng of template DNA. The samples were incubated at 65 °C on the Eppendorf ThermoMixer C with the ThermoTop for up to 60 min (see full Application Note 454).

*Assessment of LAMP technique*

To assess the viability of the LAMP technique, a second experiment was performed using a different reaction kit (Isothermal Master Mix, OptiGene) without a colorimetric dye. The LAMP reactions containing the master mix and 50 ng of template DNA were incubated at 65 °C on the Eppendorf ThermoMixer C with the ThermoTop for 45 min.

Additionally, a PCR was performed using the Mastercycler® X50a for comparison (see full Application Note 454).

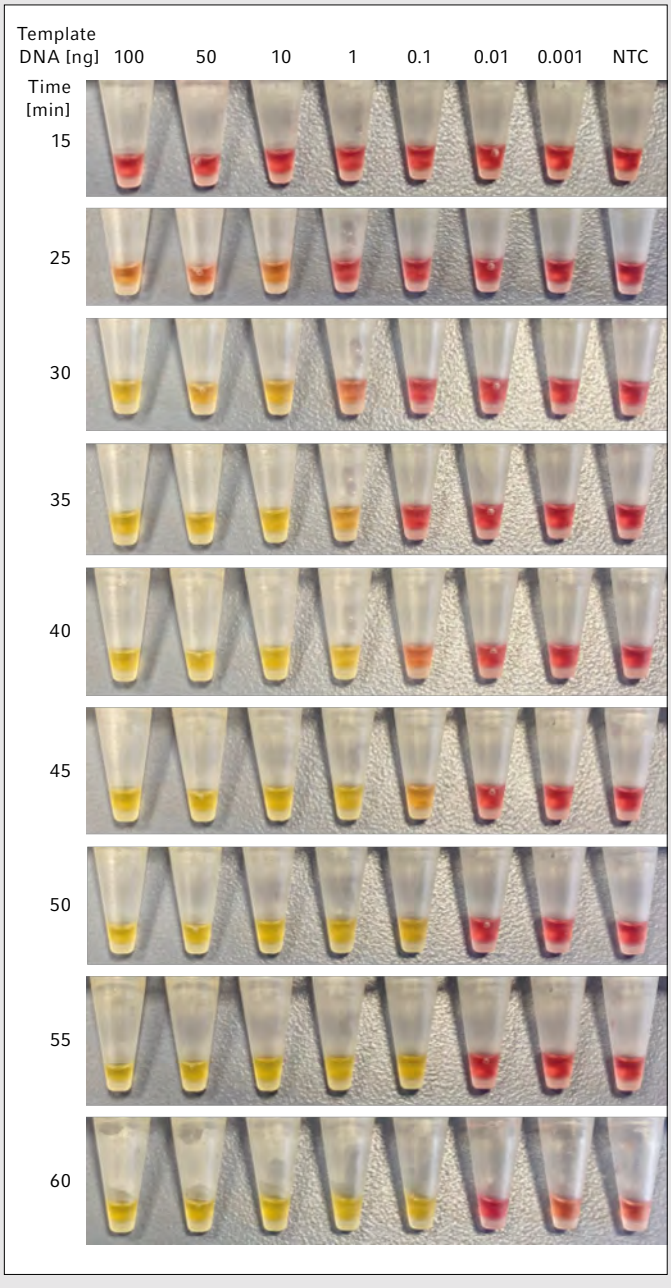


**Fig. 1:** LAMP reactions with template DNA dilutions after different incubation times

# Loop-Mediated Isothermal Amplification (LAMP) of Human Apolipoprotein L1 Gene

## Results and discussion

*Assessment of test sensitivity*

The colorimetric dye contained in the WarmStart Colorimetric LAMP 2X Master Mix allowed instant identification of amplified DNA during the incubation of the LAMP reaction. This in turn, allowed qualitative assessment of the sensitivity of the LAMP assay. As expected, the reaction took longer to change color with less template DNA (Fig. 1).

The samples with 100 ng, 50 ng, and 10 ng of template DNA were observed to begin to change color after 25 min. After 30 min, they had turned completely yellow, indicating positive detection of the *ApoL1* gene.

Under tested conditions, this protocol showed a sensitivity limit of 0.1 ng template DNA, which turned positive yellow after 45 min. The time assessment range was set at 60 min termination when the color of the no template control (NTC) was observed to have started to change, indicating that the assay was no longer reliable with further incubation.

*Assessment of LAMP technique*

To assess the viability of the LAMP technique for DNA detection, the *ApoL1* gene amplification was repeated using another reaction kit without a colorimetric dye. The results, processing times, and costs were compared to the PCR technique to analyze respective pros and cons.

Detection of the *ApoL1* gene was successfully achieved using the Isothermal Master Mix, as was shown by the characteristic smear on the gel (Fig. 2A).

The reaction took 45 min. In comparison, the PCR technique used to achieve a positive DNA amplification of the ~200 bp amplicon (Fig. 2B) took 105 min. Using fast PCR reagents and an optimized PCR protocol can further shorten the total runtime. However, an optimized LAMP kit pre-mixed with an inexpensive colorimetric dye (e.g. hydroxynaphthol blue) will be probably cheaper and definitely faster since it does not require additional equipment and saves the step of running a gel.

## Conclusion

The LAMP technique proves to be a viable method for DNA detection, as shown herein whereby the *ApoL1* gene was positively identified after 30 min. Moreover, this technique has high sensitivity of up to 0.1 ng template DNA and fast reaction completion time. Furthermore, end-point visualization of LAMP reactions can be carried out via simple colorimetric detection. This makes the additional step of running an agarose gel unnecessary, saving users the extra step and time compared to end-point PCR. In summary, the LAMP technique is a quick and simple alternative for the detection of nucleic acids that can be performed using a thermal cycler as well as an Eppendorf ThermoMixer C.

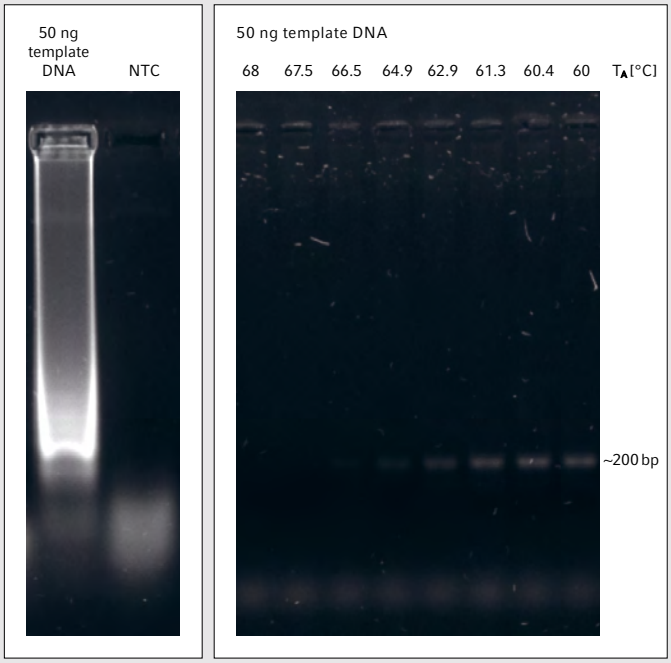


**Fig. 2: (A)** Results of the LAMP reaction performed at 65 °C using the Isothermal Master Mix without dye. The sample with 50 ng template DNA and the NTC were loaded on a 1.5 % agarose gel. **(B)** PCR with 50 ng of template DNA showed successful amplification with ca. 60–65 °C annealing temperature ($T_A$)

*Download of full Application Note 454

## Literature

[1] Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N & Hase T (2000). Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research*, 28 (12).

HANAË KÖNIG, EPPENDORF SE

# Academic Research Takes Center Stage: "Your Work Matters"

**In our current initiative "Your Work Matters", we offer scientists working in academia not only the opportunity to introduce themselves and their projects; our experts also provide tips on topics such as efficiency optimization or time savings, and they impart technological background knowledge. A forum invites participants to exchange ideas on questions concerning the laboratory – and entertainment and fun are also part of the package!**


Everyone who works in academic research suffers from similar conditions: after obtaining a university degree or completing a long apprenticeship follow long hours at work, weekend work, and low pay, paired with significant frustration, and frequently also with a lack of recognition. Presenting one's own work as well as the research results of the group are both subject to lengthy review processes in a never-ending competition for good journals. Proposals to speak at conferences, or being given the opportunity to present one's own poster, are strictly limited to the scientific data – but where is the person behind all this, and what is it that drives them?

## Your Project Matters

A platform on which one can present one's own research in a free, open, and personal manner has thus far been lacking. With "Your Project Matters", we are now giving academic research your face and your voice, presenting your research in interview-format.

In this context, we would like to ask you what compelled you to pursue a career in science and what drives you to persevere in your chosen field of research. How do you motivate yourself, your staff, and your colleagues to keep going, in the hope of improving the living conditions of people and also animals; to make new discoveries; and to prepare the world for the future?

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## Exchange and networking

In addition, you will be able to offer your opinion on questions pertaining to everyday life in the laboratory in a designated forum and find out about questions that concern other (future) researchers. Sometimes only a very small piece of the puzzle is needed to complete the whole. We are convinced that networking within an open research community is essential when it comes to harnessing synergies on a global scale. It is only hand in hand that we will be able to create the future together!

## Creating a simpler and more efficient laboratory life

Securing funding in order to increase resources or allow for conference participation requires writing numerous grant applications. How to find the time? Our experts support you in utilizing your present equipment more efficiently; in optimizing your experiments; and in the selection of the appropriate instrument with the broadest possible range of applications. Our videos, webinars, and publications are meant to make your laboratory life easier and pave the way to more time savings, more efficiency, and less frustration due to setbacks in your experiments.

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# Sustainability Report 2021: Download now

**"Improving Human Living Conditions" – as early as 1970, the founders of Eppendorf, Dr. Heinrich Netheler and Dr. Hans Hinz, formulated this visionary guiding principle for our company. Since that time, much has been achieved to ensure the fulfillment of the goals of our founders. Some 50 years later, we are shedding light on exactly what we are doing to fulfill our founders' vision – in the first Eppendorf Group Sustainability Report.**

The manufacturing of products of any kind – including those that we produce – influences the environment and the climate of our Earth. Eppendorf has declared its aim to lower $CO_2$-emissions as much as possible, which will contribute directly to our self-imposed goal of becoming neutral by 2028. The transition to 100 % renewable electricity from external sources at nearly all of our production facilities worldwide, already completed, was an important step in this direction.

Our sustainability activities are embedded in four strategic guiding principles: "Climate Change", "Natural Resources", "Social Compliance", and "Social Well-Being". It is on this basis that we as Eppendorf want to take on an industry-wide leadership role in the area of resource conservation. As a further step, we signed the "United Nations Global Compact" (UNGC) in 2022, through which we, as a part of this global network, commit ourselves to acting responsibly on the basis of the ten UNGC principles, with respect to Human Rights, Labor Standards, Environment and Climate, as well as Prevention of Corruption.

The fact that we here at Eppendorf have the potential to change things for the better is due in no small part to the close to 5,000 employees who work for the Group. We would like to thank them all for their dedication and flexibility, as well as their willingness to learn and to perform at the highest level. For the benefit of our customers, it is important to us that we conduct the entirety of our work in a sustainable fashion. Stay tuned and expect the best from us – now and in the future. We will not disappoint you!


More information on sustainability at Eppendorf:
www.eppendorf.com/sustainability

Download Sustainability Report 2021:

ANN-CLAIRE FOETSCH, ELABNEXT EPPENDORF GROUP, THE NETHERLANDS

# Is Your Laboratory Future-Proof?

**The temptation to try out a new tool for the digitalization of laboratory data is strong, and it may even be crowned with temporary success. Much can be accomplished in a shorter period of time, and many everyday tasks are easier to complete. That being said – in order to establish processes and new ways of working successfully in the long term, holistic integration is recommended. Nothing less than the future-proofing of your laboratory is at stake – with increased sample safety and greater efficiency.**


In addition to the option of instrument monitoring and alarm notification, the VisioNize Lab Suite is also ideal for the planning and allocation of daily laboratory tasks, as well as the documentation of their performance. www.eppendorf.com/visionize

Avoiding the loss of data and samples, while achieving high efficiency, are important criteria when it comes to establishing a new laboratory. Which instruments are required for daily laboratory work, and which technical specifications are relevant? Can these instruments be integrated into a complete monitoring system? What about documentation? These are the challenging questions that **Kilian Guse**, PhD, CEO and co-founder of GeneQuine Biotherapeutics, had been facing.

## In focus: sample safety and data integrity

Access to data has become much easier thanks to digitalization. Biotechnology laboratories like GeneQuine Biotherapeutics seek not only sample safety, but they also aspire to find solutions which allow flexible access to data while ensuring the continuity of laboratory operations.

The most minute temperature changes have the potential to compromise sample quality. The latest IoT* platforms (*Internet of Things) with options for remote monitoring of instruments and alarm notifications not only increase sample safety – they also uphold the confidence in laboratory data while safeguarding smooth laboratory operations.

Kilian Guse considered the interconnectivity, as well as the reading of log data, to be the most important aspects when it came to selecting and purchasing laboratory equipment. Thanks to the IoT approach and connectivity, laboratory instruments can now be networked independently of their individual manufacturers.

In this way, temperature variations in deep freezers or cell incubators can be tracked, and instrument parameters can be documented automatically and long-term on a laboratory management platform, for example, VisioNize® Lab Suite by Eppendorf.

## How to future-proof the laboratory

The Guse lab uses the VisioNizeLab Suite for the purpose of remote monitoring and alarm notifications. Its connectivity with the wide range of different Eppendorf instruments makes his laboratory as future-proof as possible. Instruments by other manufacturers can also be connected to the Lab Suite through temperature sensors.

"We have to be able to monitor and document the temperature and other parameters of our sensitive samples during long-term storage. VisioNize Lab Suite makes it possible – in a convenient and comfortable fashion", says Kilian Guse.

Watch our YouTube™ video with **Kilian Guse** on the use of the VisioNize Lab Suite at GeneQuine:

EVELYN MÖBIUS, EPPENDORF SE

# Where the Life Sciences Meet Lifestyle

**Since the first issue was published in 2016, the Eppendorf knowledge magazine "Off the Bench" has not only reported on a broad variety of topics from the world of science and research; it has also introduced the people behind the science – their stories and their perspectives.**

What's new, and surprising, in the field of science? What are the current challenges that society has to overcome? And how can everyone strengthen their own personal resilience in order to better deal with everyday life?

The knowledge magazine "Off the Bench" addresses researchers, as well as a broad audience with an interest in science. The five sections of the magazine, which is published twice a year, are conceptualized with the depth of scientific journalism.

The "Dossier" is dedicated to current and relevant topics – for example, diversity and inclusion or mental balance.


News from the world of research and interesting findings from the life sciences can be found under "Inspiring Science" and "Exploring Life". For each article, experts are consulted in order to meet the expectations of our discerning international readers. Each issue includes a trip to a cosmopolitan city – complete with suggestions for local excursions and restaurant tips.

"Bright Minds" presents exciting interviews with personalities from science and research. In the section "Inside Eppendorf", the company itself reveals innovations, new products and services, as well as current news from inside Eppendorf.

You would like to learn more? Read "Off the Bench" online, download the PDF, or subscribe to the printed magazine free of charge. Look forward to the next issue in April 2023.

More information at
www.eppendorf.com/otb

And here is the link to issue 02|22:

**News**

## Even Rockstars Need a Break

Are you working in a regulated environment? Or do your results drive critical decisions? Then you should think about servicing your pipettes regularly. They are the real "rock stars" in your lab and deserve regular breaks in an ISO 17025-accredited laboratory resort.

Calibrating pipettes in an ISO 17025-accredited calibration lab gives you peace of mind for audits and confidence in your results. ISO 17025 criteria include a traceability chain for measurement equipment, measurement uncertainty values, and approved quality standards. The service processes have been audited extensively by a regulatory authority, and the lab equipment certified by another accredited calibration laboratory.

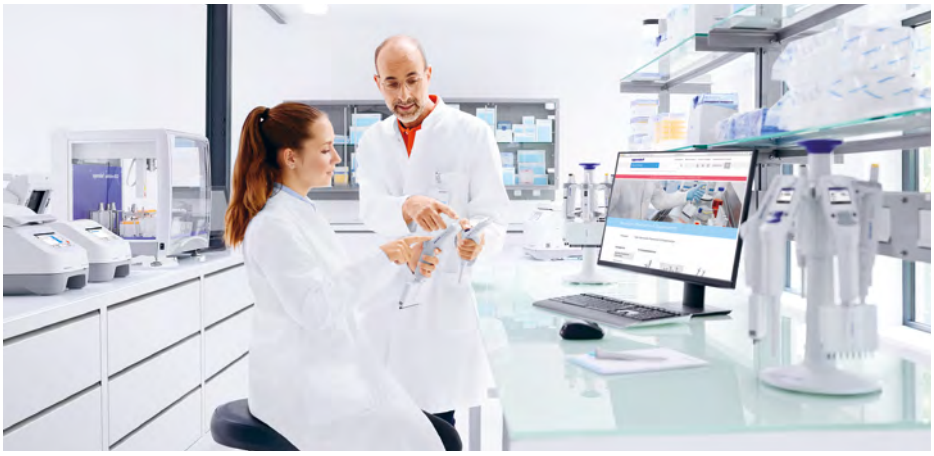
Why wait? Give your pipettes a break in an accredited laboratory resort.

Learn more in our online article!

SIMON PLATE, EPPENDORF SE

# How to Brush Up Your Pipetting Knowledge

**Pipettes are part of the basic equipment in every laboratory and are used for a wide range of applications. To get the best out of your experiments and ensure reproducible results, the right selection, as well as efficient use and proper care and maintenance of your pipetting systems are key.**



Pipettes are used for many different applications in the laboratory. Keeping up to date on current knowledge is therefore paramount

Are you a beginner, striving to become a pipetting pro? Or would you like to brush up your knowledge?

Our webinar series "Pipetting Master Class" shows in detail:

> How to select the most suitable pipetting system

> When and why an electronic pipette or a pipetting robot may be right for your application

> How to handle challenging liquids

> What to consider when it comes to the maintenance of your pipette set.

Our liquid handling experts shed a light on each one of these topics in separate episodes and offer practical tips for your daily laboratory work.

You can watch the webinars "on demand", at a flexible time of your choice, on the Eppendorf Lab Channel.

### Short and compact: our videos

For all those with less time, we recommend a new video series, featuring basic pipetting and dispensing knowledge, available on the Eppendorf YouTube™ channel. Here you can update your knowledge about liquid handling in the lab with 2–5 minute videos.

**News**

## Eppendorf Lab Channel: Already Had a Look?

The Eppendorf Lab Channel is a virtual platform where registered participants can watch live and on-demand webinars as well as new product and application demonstrations for free. Take a look over the shoulders of Eppendorf experts and get inspired by the details and insights into the lab world.

Ask questions and interact with our experts to gain new experiences up close and live. This is made possible via the chat function during the live streams. Our speakers answer directly in the live stream or after the respective presentation.

**The Eppendorf Lab Channel keeps your knowledge up to date**

The range of webinars is diverse. It covers topics such as microinjection, cell culture and PCR, as well as digitalization and liquid handling – as individual events or multi-part webinar series.

Did we pique your interest? Registration is straightforward and free of charge at www.eppendorf.com/labchannel

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CORDULA RICHTER AND CAROLYN TAUBERT, EPPENDORF SE

# Eppendorf Prize Winners on a Flying Visit to Hamburg


Kelly Nguyen, Christopher Zimmerman, Randall Platt

It's tradition that the winners of the Eppendorf research prizes are invited to visit Hamburg. Due to COVID-19, these visits had not been possible since 2020. We were therefore happy to welcome three award winners at once in Summer of 2022.

Christopher Zimmerman, USA, (*Eppendorf & Science Prize for Neurobiology* 2020), Randall Platt, Switzerland, (*Eppendorf Award for Young European Investigators* 2020), and Kelly Nguyen, United Kingdom, (*Eppendorf Award for Young European Investigators* 2022) learned interesting facts about the history of our company, the manufacture of our products, and the people at Eppendorf, both at the headquarters in Hamburg and at the production site for consumables in Oldenburg.

As in previous years, the three scientists spoke about their research to interested employees. As a farewell gift, the guests received a pipette engraved with their names, which will accompany them in their future work in the laboratory.

www.eppendorf.com/prize
www.eppendorf.com/award

eppendorf & Science PRIZE FOR NEURO BIOLOGY

EPPENDORF AWARD FOR YOUNG EUROPEAN INVESTIGATORS

https://sites.northwestern.edu/kennedylab/ann/

**Ann Kennedy wins *Eppendorf & Science Prize* 2022**

Congratulations to Ann Kennedy, Ph. D., Assistant Professor at Northwestern University, Chicago, USA on winning the 2022 *Eppendorf & Science Prize for Neurobiology*.

Brains are not simple input-output machines: we respond differently to the world we encounter depending on feelings like hunger, alertness, or anxiety. But how does the brain keep track of these signals, and how do they alter our decisions? In her own lab, Ann Kennedy collaborates with experimentalist researchers to characterize the activity of hypothalamic neurons implicated in the control of essential survival behaviors such as aggression, fear, and reproduction. While neurons in some hypothalamic nuclei have clear responses to specific behaviors, other regions show only a weak correlation with animal's actions. With other scientists, Dr. Kennedy showed that a hypothalamic nucleus implicated in defensive behaviors shows persistent activity that long outlasts the stimulus that evoked it. This activity was required to keep animals in a defensive motivational state. She went on to show how the complex responses of individual neurons gave rise at the population level to a low-dimensional signal that escalated in intensity with animals' level of aggressive motivation. This work helps us understand how our motivations and emotions emerge from the activity of our brain.

# Win an Electronic Pipette

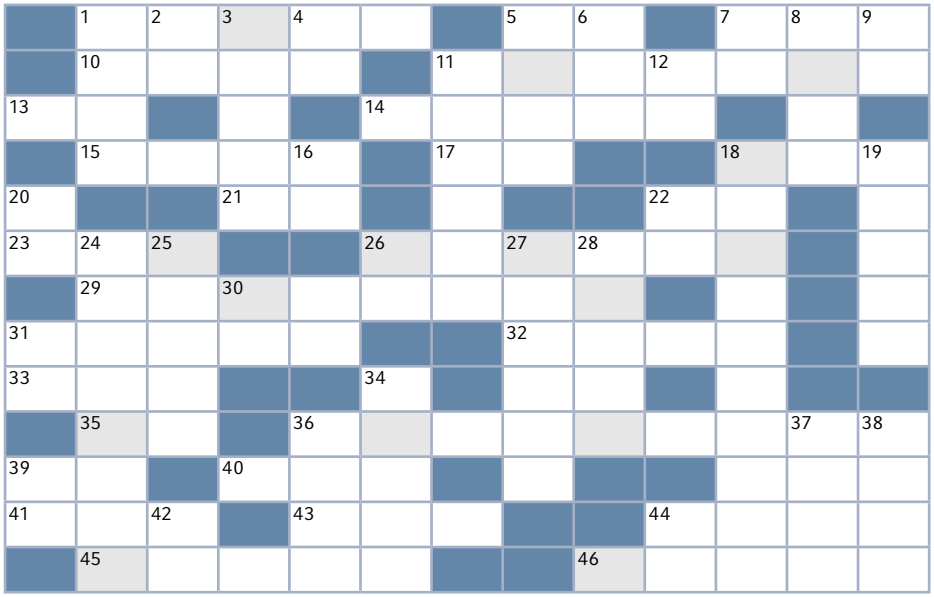
The solution of the prize competition of BioNews No. 56 was "ELABJOURNAL". The main prize, an Eppendorf Research® plus Move It® multi-channel pipette, went to Christelle N., Luxembourg.

**Good luck in our new competition!**

Simply arrange all letters in the light gray boxes of the crossword in the correct order. Send us the solution until **June 30, 2023**.

Participate online at **www.eppendorf.com/bn-service** or e-mail the solution to **bionews@eppendorf.de**.

All correct answers will be considered for a prize for use in your lab. Winners will be notified in writing. Cash payment of the prize is not possible. No recourse to legal action. The judges' decision is final. Eppendorf employees and their families may not participate. The winner of the first prize will be published in BioNews No. 60.



## 1st Prize:

1 Eppendorf Xplorer® plus 8-channel pipette of your choice

## 2nd to 5th Prize:

1 Amazon® Voucher worth 50.00 EUR

## 6th to 10th Prize:

500 bonus epPoints® each

(epPoints registration required)

### ACROSS

1 Female first name
5 Longest river in Italy
7 For, not against
10 First name (male/female)
11 French doorman
13 Home of the Lakers (abbrev.)
14 This apparatus is an organelle found in eukaryotic cells
15 Glamorous type of queen
17 Between tungsten and osmium (chem. symbol)
18 Iconic German hard rock and metal festival, est. 1990 (abbrev.)
21 Opposite of Yes
22 The capital is Mogadishu (ISO country code)
23 Nomen … omen
26 Complements an Eppendorf cycler
29 Analyst assisting police investigations
31 Mischievous trick, joke
32 Opera by Verdi
33 Name component of Eppendorf PCR plates
35 Abu Dhabi is the capital (ISO country code)
36 Venue of Nobel prize ceremonies for e.g. medicine, physics
39 The world's largest archipelagic state (ISO country code)
40 Complements Janeiro and Grande
41 Not as dry as brut (French term)
43 Unit of illuminance
44 Of … and Men (novel by John Steinbeck)
45 Performs various tasks in an automated manner
46 Sail or motor driven boat

### DOWN

1 Its chemical symbol is Pb
2 Has the atomic number 33 (chem. symbol)
3 Relating to a city
4 Radioactive alkaline earth metal (chem. symbol)
5 Best starting position
6 Generic top level domain, originally for organizations
7 Mathematical constant
8 Known for its casinos
9 Gold in French
11 Can be found in a building or on the internet
12 Transition metal, used e.g. in prostheses and implants (chemical symbol)
16 East Asian strategic board game
18 Addicted to work
19 These tubes provide light protection for samples
20 Noble gas often used in signs (chemical symbol)
22 Home of Astrid Lindgren (ISO country code)
24 Infects other people with germs
25 The next twin.tec generation
26 U.S. state in the Great Lakes region (U.S. postal abbrev.)
27 Stands for the S in SAR
28 … or treat
30 Preposition
31 Lisbon is the capital (ISO country code)
34 Type of dark, strong beer
36 Bin for storing material
37 Scottish lake
38 … and greet
39 This country's currency is ISK (ISO country code)
42 Element with the atomic number 27 (chem. symbol)
44 Mother (very short)

**Solution hint for prize competition of BioNews No. 58:**

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | R | | | R | | | A | | | | R |

Send us the solution until **June 30, 2022**. Participate online at **www.eppendorf.com/bn-service** or e-mail the solution to **bionews@eppendorf.de**.
Information about the use of your personal data can be found at **www.eppendorf.com/gdpr**

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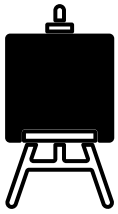

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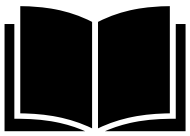

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