BIO NEWS

No. 60 – 2024

eppendorf

Mastercycler® X40: Sustainable and Ergonomic

> Bioprocess innovations for CGT development
> Your successful debut to shaken mammalian cell culture
> PCR: Zero tolerance for deviations

Comparison of the Cytotoxicity and Leaching Effects between epLIPS.® BioBased and epLIPS. Standard

Application Notes

Increasing iPSC numbers through systematic cell culture process optimization · Fast and efficient isolation of exosomes from stem cells using high-speed and ultracentrifugation · etc.

Welcome

to the latest BioNews issue. In our leading article we are presenting the new Mastercycler® X 40. With the design of this super compact PCR instrument, we have paid close attention to sustainability and ergonomics, two aspects which are gaining more and more relevance in the laboratory (pages 4–5).

CGT – cell and gene therapies – offer new opportunities for the development of treatments for previously incurable diseases. You can find out how our bioreactors contribute to this on pages 6–7.

With the acquisition of the renowned centrifuge business of Japanese Koki Holdings Co., Ltd., in 2020, Eppendorf was able to complete its centrifuge portfolio. From now on, literally no wish remains unfulfilled when it comes to centrifugation (page 9).

More than 60 years ago, the Eppendorf Tube saw the light of the laboratory world and set out to revolutionize scientific experiments and processes significantly. However, this tube – affectionately called "Eppi®"– was only the beginning. Subsequent Eppendorf Tubes® with their innovation leaps have helped to meet the increasing requirements of modern science. And there is no end in sight to our developments; only recently we have introduced the popular Eppendorf twin.tec® PCR Plates in a more sustainable "BioBased" variant or pre-labeled with "SafeCode" barcode for safe sample identification and management. Learn more on pages 12–13 and page 10.

The top prize of our competition is a set of 3 pipettes (page 15). Have fun!

We hope you'll like the second purely digital Eppendorf BioNews.
Feel free to share the link to the free online subscription.

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!

Imprint

Publisher
Eppendorf SE, Barkhausenweg 1,
22339 Hamburg, Germany
Telephone: +49 40-53801-0
Fax: +49 40-53801-556
E-mail: bionews@eppendorf.de
www.eppendorf.com/bionews

Editorial team
Berrit Hoff (Editor-in-Chief),
Dr. Jan-Hendrik Bebermeier,
Dr. Tanja Musiol, Natascha Weiß

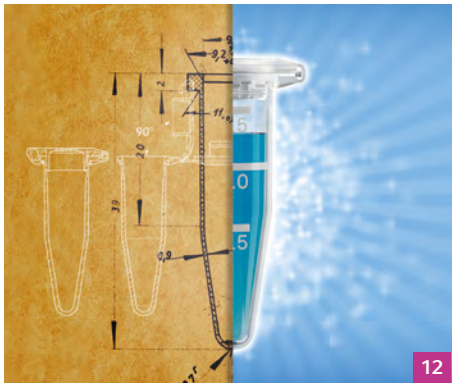
Design
Holger Paulsen Grafik-Design,
Hamburg, Germany

Image references
All images Eppendorf SE. Exception p. 14 right: Jesse Zhan Photography, USA

Important notes
We welcome all readers' articles for this publication. However, no responsibility is accepted for unsolicited manuscripts. The new products described may be launched at different times in various countries. Please contact your local Eppendorf organization or distributor for details.

Technical specifications subject to change. Errors and omissions excepted.

Eppendorf worldwide
www.eppendorf.com/contact



Eppendorf BioNews Application Notes


Comparison of the Cytotoxicity and Leaching Effects between epT.I.P.S.® BioBased and epT.I.P.S. Standard

HANAË KÖNIG, EPPENDORF SE

Mastercycler® X40 Sets New Standards for Sustainability

Sustainability is a complex and multifaceted topic which is not limited to the reduction of CO_2 emissions and waste separation. Sustainability encompasses additional aspects which may also be relevant in the laboratory. With the design of the new PCR instrument Mastercycler X40, Eppendorf paid close attention to, and incorporated into the development, several of these aspects; among them ergonomics, which also contributes to the sustainability of every person who will work with the instrument.

Ergonomics and sustainability are two of the most important topics in any laboratory. Work must be well organized, and it should be as easy as possible to carry out. This is the only way to obtain your results quickly, analyze them and then plan the next steps. At the same time, leaving the smallest possible ecological footprint in the process is becoming ever more important.

With these demands concerning ergonomics and sustainability in mind, Eppendorf developed and designed the new Mastercycler X40. Throughout this process, we have complemented our decades of expertise in the development and design of PCR machines (the first Eppendorf cycler was introduced to the market in 1990) with the latest findings with respect to ergonomic work and the reduction of energy consumption and packaging waste, as well as the utilization of resources.

Modern, clear, flexible

The result was a PCR cycler with a clear modern design which allows intuitive operation via a colored touch screen. The Peltier elements built into this instrument ensure excellent temperature homogeneity across the entire heating block.

The Mastercycler X40 heats samples in a homogeneous manner at a rate of 3.3 °C/s; cooling occurs at 1.5 °C/s. A 12-column gradient enables optimization of the temperature steps within the protocol and thus accelerates the use of new primers and a variety of DNA samples. For the transfer of protocols from slower cycler models to the Mastercycler X40, the practical "Program Migration Feature" is available which automatically adapts the heating and cooling rates to the tried-and-tested PCR protocol while maintaining the exact same run time. The Mastercycler X40 is extremely flexible: the aluminum thermoblock accommodates 0.1 mL or 0.2 mL PCR tubes or PCR tube strips as well as all types of 96-well PCR plates. The SafeLid adapts to the height of the respective tube, reliably protecting the samples from evaporation.

Tip: As a "VisioNize® touch enabled" instrument, the Mastercycler X40 can be connected directly to our cloud-based platform "VisioNize Lab Suite", for monitoring, audit trails, and documentation.

Ergonomics for a positive user experience

With respect to ergonomics, the development team has come up with an impressive array of innovations; for example, quick and intuitive programming, easy opening and closing of the lid as well as a status LED that shows from afar whether the instrument is running or not. Add the light weight of only 7.25 kg and an extremely compact format: the Mastercycler X40 is only insignificantly larger than a letter-sized piece of paper and fits perfectly onto any laboratory bench. Thanks to the ventilation slits situated at the back of the instrument, no adjacent instruments or persons will be impacted.

Mastercycler X40: super compact and easy to operate

Measurements revealed that the Mastercycler X40, at only 40.5 dB(A), is extremely quiet, and that the noises generated by heating and cooling during a PCR run will blend in with the naturally occurring background noise of a lab. This ensures a relaxed work atmosphere as laboratories with many electrical devices are often subject to high levels of noise.

Find out more on this topic in our Application Note 474.

Comprehensively sustainable

The product concept of the Mastercycler X40 is designed in a comprehensively sustainable manner. For example, the optimally coordinated components enable the very low energy consumption of only 0.134 kWh for a common 3-step PCR run. In contrast to other PCR-instruments, 129 kg CO_2 can be saved per year: see infographic.*

The SafeLid reliably protects the sample from evaporation – whether in a PCR plate or a PCR tube

With packaging, too, the emphasis was on sustainability. The Mastercycler X40 is packaged in a stable carton with 60 % recycled content; two thin recyclable plastic bags serve as dust protection. An elaborate operating manual is not included – to save paper, but not least because the instrument is so easy to operate, the Mastercycler X40 is only accompanied by a reduced short manual. If required, the comprehensive operating manual may be conveniently accessed via QR code. Even logistics were reconsidered: due to the smaller dimensions of the Mastercycler X40, twice as many instruments as before fit onto one pallet.

Conclusion

The new Mastercycler X40 not only sets new standards for PCR, but it achieves important sustainability goals. It reduces the noise level in the laboratory; it makes do with less packaging and paper, and it reduces your CO_2 footprint.

More information at eppendorf.link/raiseyourstandard

*Calculated on the basis of four PCR runs per day, on five days per week.

Source: https://www.eea.europa.eu/en/analysis/indicators/greenhouse-gas-emission-intensity-of-1

Tip

Experience the Mastercycler® X40 Live

You would like to take a close-up look at the Mastercycler X40 or other Eppendorf products and gather information in person?

The year 2024 has again many opportunities in store for you for this exact purpose: visit us at the **analytica exhibition from April 9–12, 2024 in Munich, Germany (hall B1, booth 301)** or at the **ACHEMA® in Frankfurt, Germany, from June 10–14, 2024 (hall 12.0, booth A115).**

The list of national and international events in which Eppendorf or Eppendorf subsidiaries will participate as exhibitors is long. It includes renowned events such as Forum Labo (Lyon, France), SLAS (Boston, USA), Laborama (Brussels, Belgium), WoTS (Utrecht, The Netherlands), analytica China (Shanghai, China), Medlab Middle East (Dubai, UAE), and many more. However, our presence is not limited to large exhibitions; we also participate in many regional workshops and symposia.

When and where will you find us?

Visit our event website to gain an overview of when and where you will find Eppendorf in your region. We look forward to good conversations with you!

www.eppendorf.com/events

LINA TAO, EPPENDORF SE BIOPROCESS UNIT, JUELICH, GERMANY

Bioprocess Innovations for CGT Development

CGT – cell and gene therapies – offer new opportunities for the development of life-saving treatments for previously incurable diseases. As the name suggests, they use cells or gene-modifying tools, such as stem cells, T cells, or viral vectors. CGT is a comparatively young field, and the development of these novel therapeutics still offers some challenges. Learn here how bioreactors can help scientists to meet them.

Traditionally, stem cell and CGT processes have been developed using 2D static systems, meaning cells were cultivated mostly in monolayers in cell culture flasks or dishes. This sufficed for research primarily focused on autologous applications, in which patients donate their own cells for the treatment of themselves. As the field rapidly evolves, with increasing involvement from the industry, the focus is shifting from autologous to allogeneic applications. In allogeneic therapies, healthy donor cells are modified to treat multiple patients, rather than tailoring treatment to each individual. This transition represents a notable progression in the field, as it expands the potential reach of these therapies. But this now brings more challenges as the processes need to be standardized and scalable and traditionally 2D static processes don't provide enough capacity or the expansion capability to cultivate enough cells.

Bioreactor technology from Eppendorf helps meeting these challenges in the development of cell and gene therapies.

Producing large amounts of cells

Stirred-tank bioreactors are an ideal solution to produce large cell quantities. They are scalable from a few milliliters to many liters. BioBLU® c Single-Use Bioreactors from Eppendorf, for example, cover working volumes from 100 mL to 40 L. Like this, processes can be developed and tested in small volumes, which saves resources. The process can then be transferred from small to larger volumes, even aided by innovative software solutions.

BioBLU c Single-Use Bioreactors cover working volumes from 100 mL to 40 L

Creating an optimal growth environment

3D-stirred bioreactors offer several advantages over traditional 2D cultivation platforms such as plates and flasks for cell cultivation during the development of novel therapies. One advantage is that they can provide a more physiologically relevant microenvironment for cell culture, with improved mass transfer, nutrient distribution, and waste removal through perfusion. As one result they allow for higher cell densities, which can reduce the overall time and costs for cell expansion.

Speed up development

The parallel control of multiple bioreactors enables multiple experiments to be run simultaneously. This speeds up process development, enables for process scale-out, fosters standardization, and saves costs, especially, when single-use bioreactors are used.

Save time and reduce risks

Single-use bioreactors eliminate the need for time-consuming and costly cleaning and sterilization processes associated with traditional stirred-tank bioreactors. This streamlines the workflow, reduces downtime between batches, and enables faster turnaround times.

Long-lasting expertise

Eppendorf has always been a leader in developing innovative laboratory equipment. Our expertise comes from more than 70-year history in cell culture bioprocessing and more than a decade of experience in supporting the CGT therapy market. Eppendorf Bioprocess core strength has been in bioreactor solutions at small and bench scale; we are a leading supplier in small and bench scale upstream bioprocessing. The BioBLU c Single-Use Bioreactor portfolio covers the perfect volume range needed for the development and optimization of CGT therapy processes (~60 mL) and is scalable up to 40 L, which is already the production volume, when we think of current CGT applications.

DASbox Mini Bioreactor System for cell culture

Collaborating with our customers

We take the approach of listening and understanding our customers' challenges and pain points to ensure that Eppendorf Bioprocess equipment is always at the forefront of innovation and meeting the changing needs of the market. A close collaboration with Dr. Robert Zweigerdt from the Hannover Medical School in Germany is one example. Robert is a leading scientist developing bioprocesses for the clinical translation of cells derived from human pluripotent stem cells. We worked closely with Dr. Zweigerdt and his team to develop a special 8-blade impeller, optimized for the cultivation of stem cells as aggregates in suspension. With this impeller and our DASbox® bioprocess controller, he recently achieved more than 35 million cells/mL after just seven days of cultivation.

Read Application Note 3–4 in this issue.

The collaboration with our customers is an essential part of the Eppendorf DNA. It is made possible by the experience of our sales, service, and in-field applications team that is active globally in all regions. This said, we encourage everyone to get in contact with us, for example by writing an e-mail to

bioprocess-info@eppendorf.de.

We are always open to collaborating and co-develop together with our customers.

More information

Visit our website to learn more about the Eppendorf Bioprocess solutions for cell and gene therapy development.

News

More Sustainable Liquid Handling

The Multipette®* E3 and E3x multi-dispenser pipettes, and their corresponding Combitips® advanced dispenser tips, are the latest Eppendorf products that have been awarded the prestigious ACT® Label by My Green Lab®.

ACT labels offer a transparent rating system for the sustainability of lab products, empowering laboratories to make eco-conscious choices by assessing factors such as production processes, energy usage, water consumption, packaging, and end-of-life considerations.

The Multipette/Combitips system is the first multi-dispenser system in the market to receive the ACT label. Other ACT-labeled liquid handling products from Eppendorf include Eppendorf Research® plus single-channel pipettes, Eppendorf Xplorer® and Xplorer plus electronic pipettes, and epT.I.P.S.® BioBased pipette tips. Eppendorf will continue developing products that are both cutting-edge and eco-friendly to drive positive change in the life science industry.

These products are manufactured in facilities with a strong commitment to energy efficiency, waste reduction, and responsible chemical management. Eppendorf is continuously increasing its use of recycled and recyclable packaging materials, further reducing its environmental footprint.

www.eppendorf.com/sustainability

More about My Green Lab

*U.S./CAN: Repeater®

ACT.
The Environmental Impact Factor Label

CHRISTIAN HABERLANDT, EPPENDORF SE

Your Successful Debut to Shaken Mammalian Cell Culture

Mammalian cells in suspension – either for the purpose of expressing more complex recombinant proteins, or the production of bioreactor starter cultures – are typically cultured in shake flasks. Two different culture systems are commonly used which differ significantly, particularly with respect to capacity. The aim of this article is to assist beginners in the selection of a system that is right for them.

Open platform shakers, specifically developed for use inside CO_2 incubators, represent a simple cultivation system for pilot studies and low-throughput projects, with flask volumes of up to 1 L. Platform shakers offer a cost-effective starting point if a CO_2 incubator with the required load capacity and cable port is available in the laboratory.

If, however, you are planning to increase your throughput in the future, a laboratory shaker with CO_2 control is recommended. The drives of these instruments are designed for continuous operation with a high platform load. Moreover, the stackability of these shakers ensures maximum utilization of precious laboratory space.

A seamless, fanless chamber with few mounting parts minimizes the cleaning times required. In addition, systems with an integrated high-temperature disinfection function offer powerful protection against costly contaminations that are arduous to eliminate.

Selection of the shaker system

If, after careful deliberation, you are considering purchasing an open platform shaker for CO_2 incubators as your starter instrument, bear in mind that it should meet the following minimum criteria:

> Minimal heat generation to ensure that the temperature control of the CO_2 incubator is not disturbed (risk of condensation). Ideally, the complete assembled system is tested in advance.

> Enclosed housing made from corrosion-resistant material to prevent the penetration of media and damage by the high humidity.

> The total weight, including platform load, should not exceed the maximum load capacity of the shelves of the CO_2 incubator.

> Easy cleaning with decontamination option – saves time and ensures high protection from contamination.

Preventing surprises

Consulting the instruction manual prior to purchase can save you from unpleasant surprises after the purchase.

> Is it possible that the ambient humidity in your laboratory may exceed 60 %? If yes, it is advisable to verify that the control unit of the shaker is suitable for this condition.

> You are intending to shake plates or vessels at more than 200 rpm? Ensure that the instrument is capable of providing the required speed and that the drive is designed to withstand such use over the long term.

> Is the CO_2 incubator intended for simultaneous static cultivation of cells? Check to which extent the vibrations of the shaker are transferred to the shelves – a deciding criterion when it comes to sensitive and freshly seeded cells.

Additional tips are available to you in our **White Paper 070 "CO_2 Resistant Orbital Shaker Selection and Comparison with Integrated Devices"**.

Comparison of the Cytotoxicity and Leaching Effects between epT.I.P.S.® BioBased and epT.I.P.S. Standard

JANA SCHMIDT, EPPENDORF SE, HAMBURG, GERMANY; ESTELLE DEBOEVER, EPPENDORF APPLICATION TECHNOLOGIES S.A., NAMUR, BELGIUM
JEAN-FRANÇOIS HOET, MURIEL ART, EPPENDORF CORE TEST LAB, NAMUR, BELGIUM

Abstract

In this study, the new Eppendorf epT.I.P.S. BioBased pipette tips (made from biobased feedstocks) were compared to epT.I.P.S. Standard (made from fossil fuels).

There were no significant differences noticeable between the performance of the pipette tips in any of the parameters tested. This indicates that the more sustainable, biobased material from renewable feedstocks offers the same properties as materials derived from fossil-based sources.

Introduction

A pipette and a pipette tip comprise a system which ensures precise results in any laboratory procedure involving liquid handling steps. Whereas the pipette can be used for years, the plastic tip belongs to the single-use consumables which contribute to laboratory waste, and which must be taken into consideration with respect to the quest for more sustainability and reduced reliance on fossil raw materials.

Eppendorf Tubes® were the first lab consumables manufactured from biobased feedstocks, opening the door for more sustainable labware. They are now followed by the epT.I.P.S. BioBased pipette tips. These are manufactured from a minimum of 90 % renewable feedstocks and thus significantly reduce the use of fossil resources required for the production of the products.

This study compares epT.I.P.S. BioBased and epT.I.P.S. Standard in the Biopur® purity with respect to the parameters cytotoxicity and leaching in order to examine whether the new source of raw material exhibits comparable properties to those of the standard material. Competitors' tips were also tested for the parameter leaching.

Materials and methods

Materials

> epT.I.P.S. BioBased Biopur, Reloads, 2–200 µL
> epT.I.P.S. Standard Biopur, Racks, 2–200 µL
> Pre-sterilized 200 µL non-filtered pipette tips from other manufacturers

For a complete description of the materials and methods used, see **Application Note 477**.

Cytotoxicity assay

Preparation of the liquid extract

The pipette tips tested were cut into small pieces, placed in extraction vessels, and covered with complete medium (4 mM MEM glutamine, 100 UI/mL penicillin, 100 µg/mL streptomycin, 10 % FBS) in a 3 cm^2/mL surface-to-volume ratio. The extraction conditions were 37 °C for 72 h, compliant with ISO standards 10993-5:2009 and 10993-12, and additional extracts were generated at 50 °C for 24 h and 37 °C for 30 min. Following the incubation, the extracts were used for cell culture growth of murine L929 fibroblasts (N = 3).

Cell viability – morphology

L929 cells were cultured in complete medium (ATCC®, 30-2003; 5 % CO_2) and digested by 25 % trypsin/EDTA to obtain a single-cell suspension. After inactivation of the trypsin/EDTA, cells were collected and diluted in fresh medium to achieve a cell density of 1×10^5 cells/mL. After 48 h, cell morphology was examined, and parameters including detachment, cell lysis, and vacuolization were assessed in accordance with the ISO 10993 standards.

Cell viability – MTT assay

After the morphology assessment, the medium was replaced with an MTT solution (1 mg/mL; 50 µL/well). The cells were incubated for 2 h (37 °C, 5 % CO_2). Absorbance of the culture at 570 nm indicated its viability, as only intact, metabolically active cells are capable of converting the yellow MTT dye to the purple metabolite. Cells cultured in untreated medium served as control.

Leaching

One pipette tip was placed inside a glass tube and fully covered with 8 mL ethanol, 99.9 % p.a. The glass tube was sealed with aluminum foil and incubated inside a shaker at a 45° angle (60 °C, 140 rpm). Following a defined time interval, 200 µL of the ethanol were transferred directly to an Eppendorf UVette® and absorbance was measured at 260 nm and 280 nm. Ethanol 99.9 % p.a. incubated in the same manner without a tip served as a blank (N = 3).

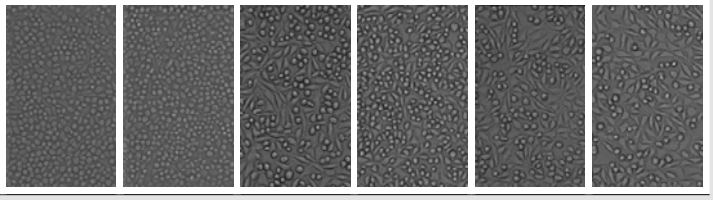
Extraction conditions		37 °C 30 min		37 °C 72 h		50 °C 24 h	
epT.I.P.S.		Standard	BioBased	Standard	BioBased	Standard	BioBased
Replicate	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0
Morphology							

Fig. 1: Evaluation of the morphology of L929 cells after growth in medium which, in accordance with ISO 10993, had been pre-incubated with pipette tips. A score below 2 is indicative of non-cytotoxic material. Neither epT.I.P.S. BioBased nor epT.I.P.S. Standard show any cytotoxic effects on the murine fibroblasts

Results and discussion

Cytotoxicity

Murine L929 cells were grown in media which had previously been incubated with the respective pipette tips in compliance with ISO standard 10993. Culture morphology was assessed in a qualitative manner (Fig. 1).

Comparison of the Cytotoxicity and Leaching Effects between epT.I.P.S.® BioBased and epT.I.P.S. Standard

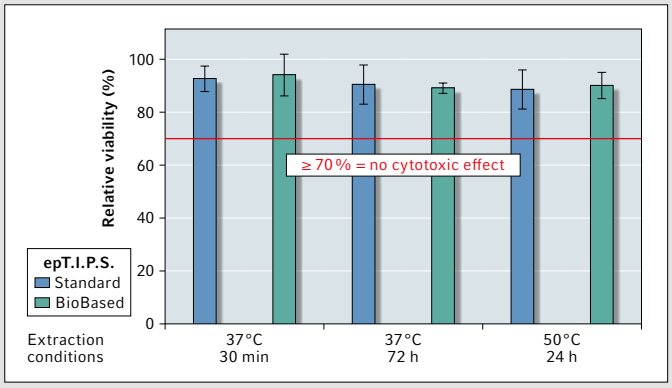


Fig. 2: Relative viability of L929 cells as determined by MTT assay, in accordance with ISO 10993. A value above 70 % shows that the tested material does not elicit cytotoxicity. Neither epT.I.P.S. BioBased nor epT.I.P.S. Standard show any cytotoxic effect

A score of 0 indicates no abnormalities whereas scores greater than 2 are indicative of cytotoxic effects. The MTT assay was used as a quantitative parameter for the viability of the cells (Fig. 2).

A relative viability of more than 70 % indicates a non-cytotoxic material. Neither case showed any cytotoxic effects of the pipette tips, and no differences between the two types of pipette tip could be detected.

Leaching effects

Compared to reaction vessels, pipette tips are in contact with the sample for a short time only. Nevertheless, concerns arise with respect to substances leaching from the plastic ("leachables"), especially during work with organic solvents. For this reason, the tips, as well as comparable products by competitors, were incubated in ethanol, and the ethanol extract was subsequently measured photometrically to test for leachables. In general, leachables show absorbance in the UV-range. Since biochemical analytic assays are also performed at 260 nm and 280 nm (quantification of DNA and protein, respectively), these leaching effects have significant potential for interference.

Even after incubation for 24 h, very little leaching was detectable from both variants of the epT.I.P.S. Comparable pipette tips by competitors showed noticeably elevated leachable effects in both wavelengths after only 1 h of incubation (Fig. 3).

Conclusion

In compliance with ISO 10993-5:2009 ("Testing for in vitro Cytotoxicity") and ISO 10993-12 ("Sample Preparation and Reference Materials"), the material cytotoxicity of the Eppendorf epT.I.P.S. BioBased, in comparison with epT.I.P.S. Standard, was assessed. Neither the fossil-based nor the biobased materials induced morphological changes, and neither material compromised cell viability. As well, the assessment of leaching effects after contact with an organic solvent showed comparable, low values for both types of pipette tip, whereas competitors' products showed noticeably higher effects.

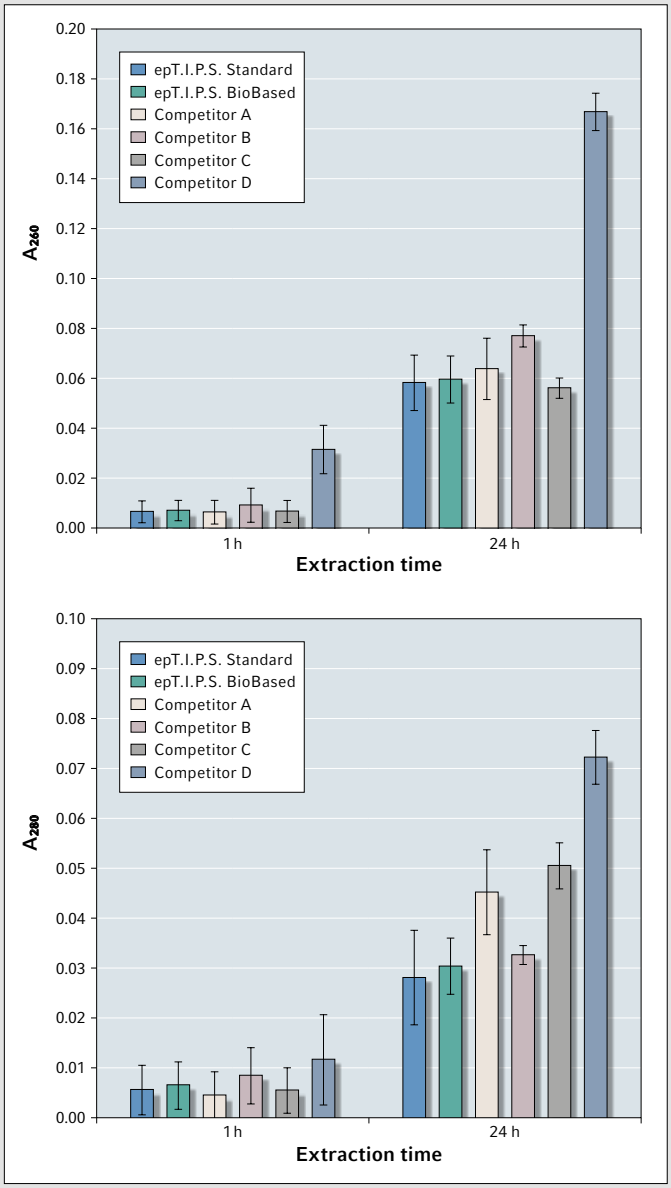


Fig. 3: Absorbance of pipette tip extracts in ethanol at 260 nm (above) and 280 nm (below) for the purpose of non-specific detection of leachables. epT.I.P.S. show the lowest absorbance values. Competitors A–D show noticeably higher absorbance values

The outstanding characteristics and suitability for biochemical applications of both Eppendorf materials – standard and biobased – could thus be verified, as well as the fact that these materials are superior to those used by competitors.

Download the complete Application Note 477

Literature

[1] www.iscc-system.org

[2] Grzeskowiak *et al.*, Eppendorf Application Note 470

Increasing iPSC Numbers through Systematic Culture Process Optimization

MANSTEIN F[1,2], ULLMANN K[1,2], KROPP C[1,2], HALLOIN C[1,2], TRIEBERT W[1,2], FRANKE A[1,2], FARR CM[1,2], SAHABIAN A[1,2], HAASE A[1,2], BREITKREUZ Y[3], PEITZ M[3,4], BRÜSTLE O[3], KALIES S[5,6], MARTIN U[1,2], OLMER R[1,2], ZWEIGERDT R[1,2]
[1]LEIBNIZ RESEARCH LABORATORIES FOR BIOTECHNOLOGY & ARTIFICIAL ORGANS, HANNOVER, [2]REBIRTH CLUSTER OF EXCELLENCE, HANNOVER, [3]INST. OF RECONSTRUCTIVE NEUROBIOLOGY, BONN, [4]CELL PROGRAMMING CORE FACILITY, BONN, [5]INST. OF QUANTUM OPTICS, HANNOVER, [6]LOWER SAXONY CENTRE FOR BIOMEDICAL ENGINEERING, IMPLANT RESEARCH & DEVELOPMENT, HANNOVER; GERMANY
CONTACT: BIOPROCESS-EXPERTS@EPPENDORF.COM

Introduction

Human induced pluripotent stem cells (hiPSCs) are a powerful tool for drug discovery, *in vitro* disease modelling, or regenerative therapies. However, such procedures require high cell numbers to be sufficiently applicable, a criterion that is hard to satisfy with traditional 2D culture methods. Stirred-tank bioreactors offer a scalable 3D culture environment suitable to provide, control, and maintain optimal growth conditions for the cell type of choice.

Fig. 1: DASbox Mini Bioreactor System

In this study, the DASbox® Mini Bioreactor System (Fig. 1) was utilized to systematically optimize process parameters of a hiPSC culture in a step-by-step process.

This approach led to a more than 10-fold increase in cell density (almost 35 × 10^6 cells/mL) compared to uncontrolled conditions while stem cell features and viability were retained.

Materials and methods

You find a complete description of the materials and methods in **Application Note 472** [1].

The cell culture experiments were conducted using three different hiPSC lines [1].

Fig. 2 summarizes the experimental procedure for iPSC culture parameter optimization: Prior to the bioreactor experiments, the cells were expanded as a feeder-free monolayer culture in flasks at 37 °C. To inoculate the bioreactor, a single-cell suspension was achieved by detaching the cell monolayer. Stem cell culturing and culture optimization was performed in DASbox Mini Bioreactors equipped with an 8-blade impeller (60° pitch) optimized for stem cell expansion, with an overhead drive for agitation, pH and DO sensors, as well as temperature control in order to ensure precise regulation of critical process parameters.

Perfusion operation mode was enabled by an outflow filter device, allowing medium to flow in and out of the bioreactor while retaining the cells inside. The experiments in bioreactors were performed as stem cell aggregate culture without the use of scaffolds for cell attachment.

Results

To increase cellular yields, the DASbox Mini Bioreactor System was employed to introduce precise monitoring and

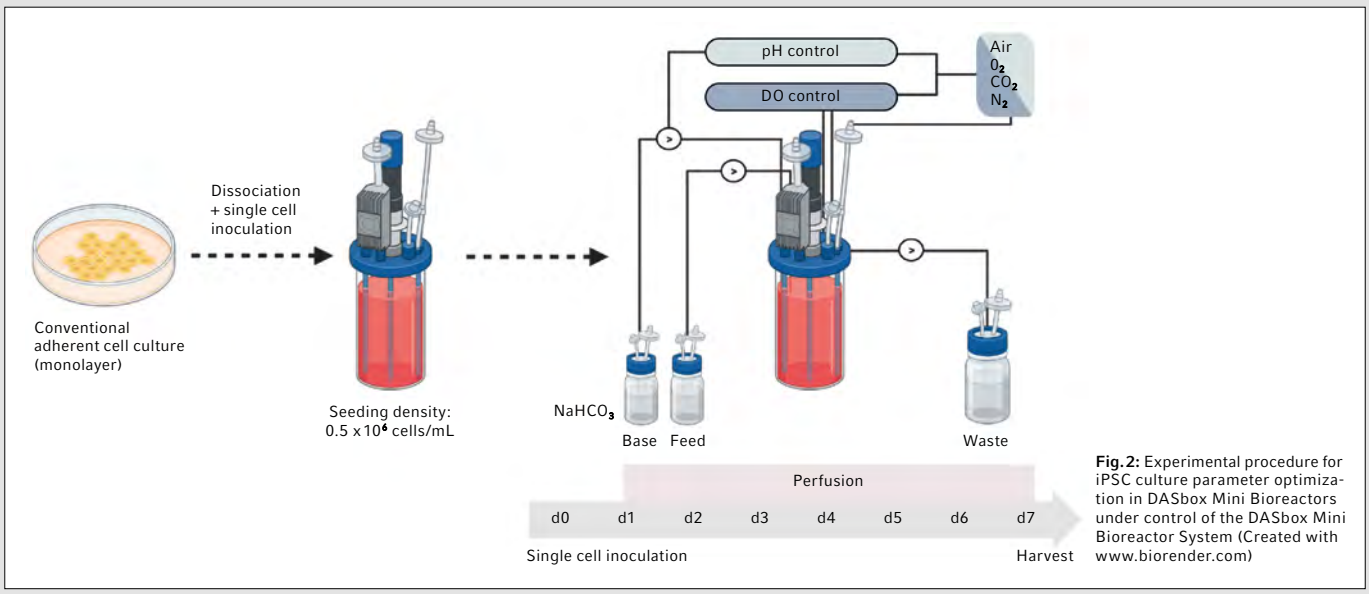


Fig. 2: Experimental procedure for iPSC culture parameter optimization in DASbox Mini Bioreactors under control of the DASbox Mini Bioreactor System (Created with www.biorender.com)

Increasing iPSC Numbers through Systematic Culture Process Optimization

control over pH, glucose feeding, and DO to an otherwise uncontrolled setting. Fig. 3 gives an overview of the process optimization steps along with the achieved cell densities.

I) Cells were cultivated in bioreactors for 7 days under **uncontrolled conditions**.

II) pH control with a setpoint of pH 7.0 was initiated once a certain pH threshold was undercut, resulting in a steady culture pH value of 7.0 throughout the run while higher fluctuations and a generally lower pH were present in the uncontrolled setting. The pH-controlled culture displayed a much higher glucose consumption over time compared to the uncontrolled setting.

III) Glucose feeding was initiated from day 3 onwards in addition to the pH control, to compensate for the decrease of this important carbohydrate source. This resulted in higher culture glucose concentrations but also increased levels of lactate, a growth-inhibiting by-product of anaerobic glycolysis.

IV) DO control was introduced with a setpoint of 40 % starting from the first day of culture to provide a steadier oxygen supply. This yielded only about the same cell numbers as the glucose-supplemented culture alone. This could be due to a lower cell count in the first days of culture. The lower cell count could be explained by the fact that in the DO-controlled run (IV) the DO concentration in the first days was lower than in the previous runs (I-III). Also, the employed DO strategy resulted in larger cell aggregates by the end of the run which can negatively impact cell viability.

V) Cell aggregate size and viability were optimized by shortening the pre-culture phase, introducing a DO-level cascade, adding a shear protectant during inoculation, and increasing the agitation speed.

VI) Feeding optimization: To enable increasing glucose levels and to prevent the rise of lactate to cell growth-inhibiting levels, the medium perfusion rate was stepwise increased from 1 to 2 culture-volumes/day between culturing day 2 and 5. At the same time, glucose concentration was increased from 3.15 to 6.15 g/L between day 1 and 3 to 6.15 to 7.65 g/L from day 4 onwards. Cell density increased once more to 18×10^6 cells/mL, almost double the amount achieved during the last optimization step.

VII/VIII) Further culture optimization by *in silico* modelling: To further push the achievable cell density and to reduce the workload of testing each parameter adaptation in a wet lab setting, culture optimization was next supported by *in silico* modelling. Wet lab results were fed into an algorithm to predict further parameter optimization approaches. This led to unmatched numbers of almost 5×10^9 cells in 150 mL culture volume, which were confirmed by using three different stem cell line culture runs under the model VIII conditions with cell viabilities comparable to previous runs.

Stem cell properties of hiPSCs cultured in a stirred-tank bioreactor

After 7 days of culture the cells were tested for pluripotency marker expression and for their differentiation capabilities. Together, the results suggest that the pluripotent stem cell population maintained all expected key properties after cultivation to high cell densities achieved by process optimization in a stirred-tank bioreactor [1].

Conclusion

Using the precise parameter control capabilities of the DASbox Mini Bioreactor System in combination with systematic adaptation and *in silico* process modelling enabled the increase of stem cell densities more than tenfold, compared to an uncontrolled setting, to almost 35×10^6 cells/mL. This demonstrates the power of a controllable and adjustable growth environment. The procedure described here can act as a roadmap to identify and overcome cultivation bottlenecks, increase stem cell numbers, and advance the field of stem cell applications.

Literature

[1] Manstein *et al.* Increasing iPSC Numbers through Systematic Culture Process Optimization. Eppendorf Application Note 472. 2023.

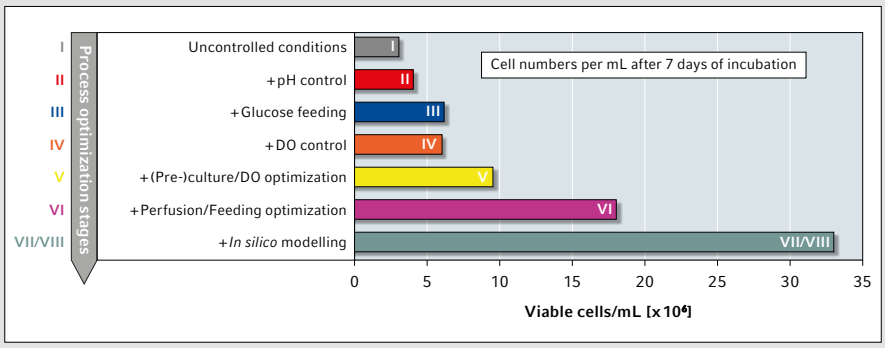


Fig. 3: Summary of the process optimization steps along with the achieved cell densities

epMotion® Method Overview

The epMotion family of liquid handling workstations allows you to easily automate reagent kits and standardized protocols which can be tedious, time-consuming, and error-prone when performed manually. In addition, automating your workflow can free up time for other tasks, increase your reproducibility, standardize your processes, and increase your throughput.

Based on two decades of experience in liquid handling automation and our expert applications support and service, a multitude of kits have been successfully automated with guidance from manufacturers or implemented directly at a customer site. As the needs of epMotion users vary, each workflow requires careful optimization, customization, and thorough training to achieve consistent, high-quality results.

Find your kit

Visit **epMotion Method Overview** to find your kit of choice from a variety of different vendors including Agilent®, CareDX®, Geneaid, 10x Genomics®, Illumina®, Macherey-Nagel®, New England Biolabs®, Omega Bio-Tek, Omixon, One Lambda, Oxford Nanopore Technologies, Promega®, QIAGEN®, Roche®, Swift BioSciences, Takara, Thermo Fisher Scientific® und Zymo Research.

If your kit of choice is not listed yet, our team of local automation specialists is happy to support you in implementing and optimizing new methods and workflows on your epMotion.

Contact us!

More information on automation

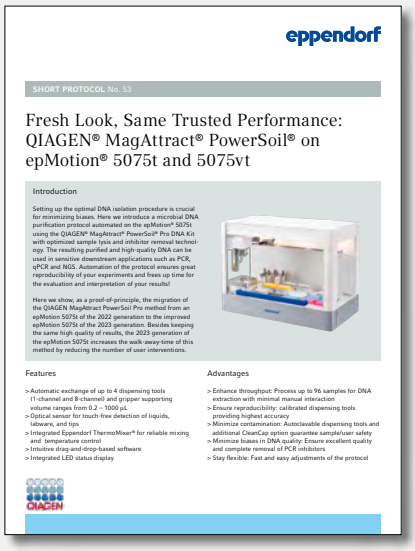
Fresh Look, Same Trusted Performance

Automated workflows on the new generation of epMotion®

Short Protocol No. 53

Isolating high-quality DNA for sensitive downstream analyses

Setting up an automated process for purification of microbial DNA from different sample sources using the epMotion 5075 and a QIAGEN kit.


eppendorf

SHORT PROTOCOL No. 53

Fresh Look, Same Trusted Performance: QIAGEN® MagAttract® PowerSoil® on epMotion® 5075t and 5075vt

Introduction

Setting up the optimal DNA isolation procedure is crucial for minimizing biases. Here we introduce a microbial DNA purification protocol automated on the epMotion® 5075t using the QIAGEN® MagAttract® PowerSoil® Pro DNA Kit with optimized sample lysis and inhibitor removal technology. The resulting purified and high-quality DNA can be used in sensitive downstream applications such as PCR, qPCR and NGS. Automation of the protocol ensures great reproducibility of your experiments and frees up time for the evaluation and interpretation of your results!

Here we show, as a proof-of-principle, the migration of the QIAGEN MagAttract PowerSoil Pro method from an epMotion 5075t of the 2022 generation to the improved epMotion 5075t of the 2023 generation. Besides keeping the same high quality of results, the 2023 generation of the epMotion 5075t increases the walk-away-time of this method by reducing the number of user interventions.

Features

> Automatic exchange of up to 4 dispensing tools (1-channel and 8-channel) and gripper supporting volume ranges from 0.2 – 1000 µL
> Optical sensor for touch-free detection of liquids, labware, and tips
> Integrated Eppendorf ThermoMixer® for reliable mixing and temperature control
> Intuitive drag-and-drop-based software
> Integrated LED status display

Advantages

> Enhance throughput: Process up to 96 samples for DNA extraction with minimal manual interaction
> Ensure reproducibility: calibrated dispensing tools providing highest accuracy
> Minimize contamination: Autoclavable dispensing tools and additional CleanCap option guarantee sample/user safety
> Minimize biases in DNA quality: Ensure excellent quality and complete removal of PCR inhibitors
> Stay flexible: Fast and easy adjustments of the protocol

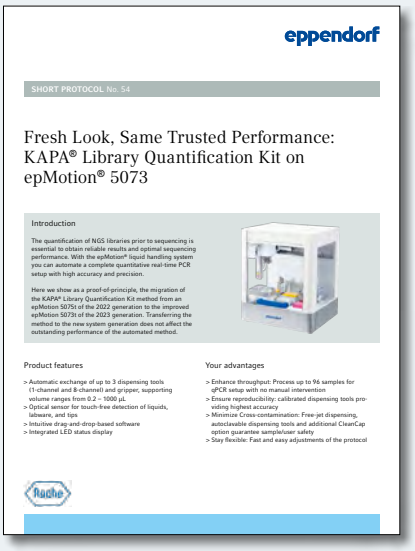
QIAGEN

Download

Short Protocol No. 54

Automated qPCR for quantification of NGS libraries

Optimal sequencing performance relies on accurate and precise qPCR results. The epMotion 5073 and the KAPA® Library Quantification Kit from Roche are a perfect combination.


eppendorf

SHORT PROTOCOL No. 54

Fresh Look, Same Trusted Performance: KAPA® Library Quantification Kit on epMotion® 5073

Introduction

The quantification of NGS libraries prior to sequencing is essential to obtain reliable results and optimal sequencing performance. With the epMotion® liquid handling system you can automate a complete quantitative real-time PCR setup with high accuracy and precision.

Here we show as a proof-of-principle, the migration of the KAPA® Library Quantification Kit method from an epMotion 5075t of the 2022 generation to the improved epMotion 5073t of the 2023 generation. Transferring the method to the new system generation does not affect the outstanding performance of the automated method.

Product features

> Automatic exchange of up to 3 dispensing tools (1-channel and 8-channel) and gripper, supporting volume ranges from 0.2 – 1000 µL
> Optical sensor for touch-free detection of liquids, labware, and tips
> Intuitive drag-and-drop-based software
> Integrated LED status display

Your advantages

> Enhance throughput: Process up to 96 samples for qPCR setup with no manual intervention
> Ensure reproducibility: calibrated dispensing tools providing highest accuracy
> Minimize Cross-contamination: Free-jet dispensing, autoclavable dispensing tools and additional CleanCap option guarantee sample/user safety
> Stay flexible: Fast and easy adjustments of the protocol

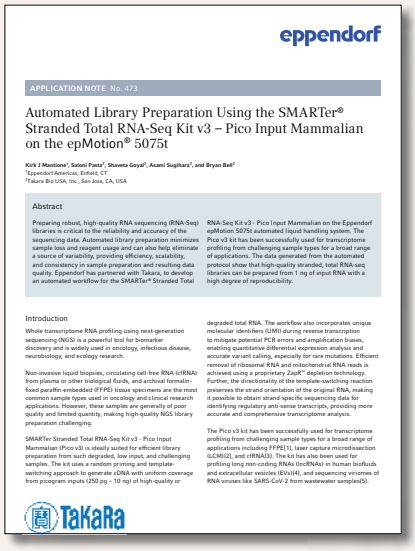
Roche

Download

Application Note No. 473

Preparing robust, high-quality RNA sequencing libraries

We developed an automated workflow on the epMotion 5075 to generate stranded, total RNA-seq libraries using a kit from Takara.


eppendorf

APPLICATION NOTE No. 473

Automated Library Preparation Using the SMARTer® Stranded Total RNA-Seq Kit v3 – Pico Input Mammalian on the epMotion® 5075t

Kirk J Mantione[1], Saloni Pasta[2], Shweta Goyal[2], Asami Sugihara[2], and Bryan Bell[2]
[1]Eppendorf America, Enfield, CT
[2]Takara Bio USA, Inc., San Jose, CA, USA

Abstract

Preparing robust, high-quality RNA sequencing (RNA-Seq) libraries is critical to the reliability and accuracy of the sequencing data. Automated library preparation minimizes sample loss and reagent usage and can also help eliminate a source of variability, providing efficiency, scalability, and consistency in sample preparation and resulting data quality. Eppendorf has partnered with Takara, to develop an automated workflow for the SMARTer® Stranded Total RNA-Seq Kit v3 - Pico Input Mammalian on the Eppendorf epMotion 5075t automated liquid handling system. The Pico v3 kit has been successfully used for transcriptome profiling from challenging sample types for a broad range of applications. The data generated from the automated protocol show that high-quality stranded, total RNA-seq libraries can be prepared from 1 ng of input RNA with a high degree of reproducibility.

Introduction

Whole transcriptome RNA profiling using next-generation sequencing (NGS) is a powerful tool for biomarker discovery and is widely used in oncology, infectious disease, neurobiology, and ecology research.

Non-invasive liquid biopsies, circulating cell-free RNA (cfRNA) from plasma or other biological fluids, and archival formalin-fixed paraffin-embedded (FFPE) tissue specimens are the most common sample types used in oncology and clinical research applications. However, these samples are generally of poor quality and limited quantity, making high-quality NGS library preparation challenging.

SMARTer Stranded Total RNA-Seq Kit v3 - Pico Input Mammalian (Pico v3) is ideally suited for efficient library preparation from such degraded, low input, and challenging samples. The kit uses a random priming and template-switching approach to generate cDNA with uniform coverage from picogram inputs (250 pg - 10 ng) of high-quality or degraded total RNA. The workflow also incorporates unique molecular identifiers (UMI) during reverse transcription to mitigate potential PCR errors and amplification biases, enabling quantitative differential expression analysis and accurate variant calling, especially for rare mutations. Efficient removal of ribosomal RNA and mitochondrial RNA reads is achieved using a proprietary ZapR™ depletion technology. Further, the directionality of the template-switching reaction preserves the strand orientation of the original RNA, making it possible to obtain strand-specific sequencing data for identifying regulatory anti-sense transcripts, providing more accurate and comprehensive transcriptome analysis.

The Pico v3 kit has been successfully used for transcriptome profiling from challenging sample types for a broad range of applications including FFPE[1], laser capture microdissection (LCM)[2], and cfRNA[3]. The kit has also been used for profiling long non-coding RNAs (lncRNAs) in human biofluids and extracellular vesicles (EVs)[4], and sequencing viromes of RNA viruses like SARS-CoV-2 from wastewater samples[5].

TaKaRa

Download

Article from BioNews 59

epMotion®: Gain Freedom to Do What Really Counts

Learn more about the new generation of epMotion and the benefits of automated workflows.


4 IN THE SPOTLIGHT · EPMOTION®: GAIN FREEDOM TO DO WHAT REALLY COUNTS

BERRIT HOFF, EPPENDORF SE

epMotion®: Gain Freedom to Do What Really Counts

In our leading article, we invite you to get to know the epMotion, one of the most precise systems for automated liquid handling on the market. If you are striving for maximum reproducibility in your assays and the highest possible flexibility in adapting to changing experimental requirements, read our interview with Dr. Tim Schommartz, Global Marketing Manager. Tim is a molecular biologist himself (with a focus on virology), and he knows from his own experience about the potential that can be unleashed for actual research through the use of automated systems.

With our epMotion systems, you will gain time and mental freedom for your true passion – research.

BioNews: Tim, February 2023 saw the worldwide debut of the new generation of epMotion at the SLAS conference in San Diego, CA, USA. Your conclusions?*

Tim Schommartz: The SLAS is basically "the place to be" for automated systems, and we were completely thrilled to be on site at this important event.

Our motto "Unleash Your Potential", too, hit the bullseye. When it comes down to it, it's about nothing else! With our epMotion systems, researchers gain freedom with respect to both time and creativity, and they can now realize their full potential for their true passion – research.

BN: By handing tedious tasks off to a "robot"?

TS: Exactly – it's about the switch from the manual to the automated workflow. We aim to show how easy and intuitive it is to implement our epMotion systems for routine tasks, in particular to those users who have not yet had a chance to familiarize themselves with laboratory automation. Even in smaller laboratories and with lower throughput, it can be done in a short period of time and without the need for extensive training!

BN: What is it that distinguishes the new generation of epMotion?

TS: The new instruments represent an advancement of the epMotion 5073 and epMotion 5075 series which have been established for nearly 20 years. These had been continually improved over time – with a focus primarily on technological

Download

Fast and Efficient Isolation of Exosomes from Stem Cells Using a Combination of High-Speed and Ultracentrifugation

PASCAL ROWART, VINCENT DUFEY, FRANÇOISE DE LONGUEVILLE, EPPENDORF APPLICATION TECHNOLOGIES S.A., NAMUR, BELGIUM
JAN KNOP, EPPENDORF SE, HAMBURG, GERMANY
CORRESPONDING AUTHOR: ROWART.P@EPPENDORF-EAT.BE

Introduction

Most eukaryotic cells release membrane-derived vesicles, also called extracellular vesicles (EVs). EVs, with sizes ranging from 30 nm to 1,000 nm, are released by numerous cell types in the extracellular space. They are spherical cytosol fragments surrounded by lipid-bilayer membranes and hydrophilic proteins, and composed of various bioactive molecules, including RNAs, DNAs, proteins, mRNA, microRNAs, and lipids. EVs are a heterogeneous group of vesicles known as exosomes (30–150 nm) and microvesicles (MVs, 150–1,000 nm).

Here, exosome isolation was carried out by the Centrifuge CR22N and Ultracentrifuge CP100NX after culturing human adipose-derived stem cells (hADSCs) in suspension on microcarriers in the DASbox® Mini Bioreactor System equipped with BioBLU® 0.3c Single-Use Bioreactors. This fully controlled system can expand the production of high-yielding viable cells and optimize the production of high-quality and high quantities of exosomes (Fig. 1).

Materials and methods

Culture of hADSCs on microcarriers in BioBLU 0.3c Single-Use Bioreactors

Human adipose-derived stem cells were expanded on T75 flasks. After 5 days, cells were mixed with microcarriers and cultured in suspension using BioBLU 0.3c Single-Use Bioreactors in the DASbox Mini Bioreactor System. On day 7, the cell culture-conditioned media (CCM) was collected for serial centrifugation steps.

Isolation of exosomes by ultracentrifugation

CCM was collected and distributed in 50TC and 15TC tubes and centrifuged for each case in the Centrifuge CR22N coupled with the Rotor R15A at 500 x *g* for 10 min at 4 °C. The supernatant was transferred to a new tube and centrifuged a second time at 2,000 x *g* for 10 min at 4 °C. The supernatant was transferred again in a new tube and finally centrifuged a third time at 20,000 x *g* for 20 min at 4 °C (Fig. 2, A).

CCM was transferred into 40PET tubes directly (Fig. 2, B) or on top of 4 mL of 30 % sucrose solution (Fig. 2, C). Tubes were centrifuged at 100,000 x *g* for 90 min at 4 °C. The supernatant was discarded. Additional CCM or PBS (depending on the initial volume of CCM) was slowly added on top and centrifuged again at 100,000 x *g* for 90 min at 4 °C. Exosomes were resuspended and stored in an aliquot at –80 °C for further use.

Size distribution analysis

The size distribution profile of EVs was measured by Dynamic Light Scattering (DLS).

A Zetasizer® Low Volume Disposable Sizing Cell Kit (Malvern®) was used to analyze exosome pellets in transparent cuvettes.

Electron microscopy

Exosome suspension solution was loaded on transmission electron microscope (TEM) grids and incubated for 1 h. Exosomes were stained with filtered 2.5 % uranyl acetate solution on the surface of the TEM grid by syringe for 10 min. Uranyl acetate excess solution on the grid was removed by contacting the grid edge with filter paper. Observations were made with a JEOL transmission electron microscope at 80 kV.

Exosome quantification by CD63 ELISA

Exosome quantity was measured using the ExoELISA-ULTRA Complete Kit (CD63 detection) following the manufacturer's instructions.

Results and discussion

Exosome isolation from the conditioned medium using serial centrifugations

The first three centrifugation steps were used to remove live cells and beads (500 x *g*), cell debris, dead cells, apoptotic bodies (2,000 x *g*), and microvesicles (20,000 x *g*) using the Rotor R15A. Using two runs of Ultracentrifuge CP100NX and Rotor P32ST coupled with a sucrose cushion, an intact and homogeneous population of exosomes was collected.

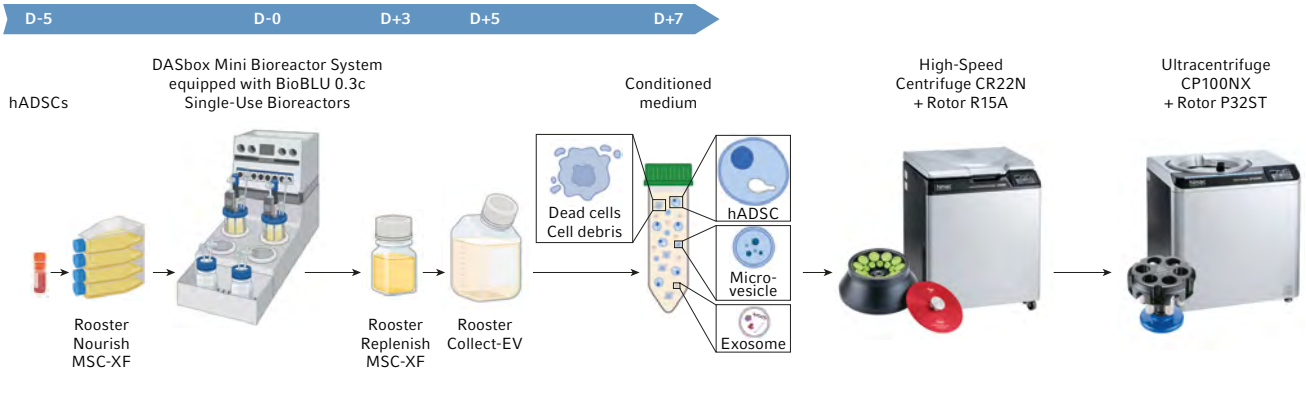


Fig. 1: Schematic representation of cell expansion, exosome production in DASbox Mini Bioreactor System equipped with BioBLU 0.3c Single-Use Bioreactors and exosome isolation using a combination of Centrifuge CR22N with Rotor R15A and Ultracentrifuge CP100NX with Rotor P32ST. Created with www.biorender.com

Fast and Efficient Isolation of Exosomes from Stem Cells Using a Combination of High-Speed and Ultracentrifugation

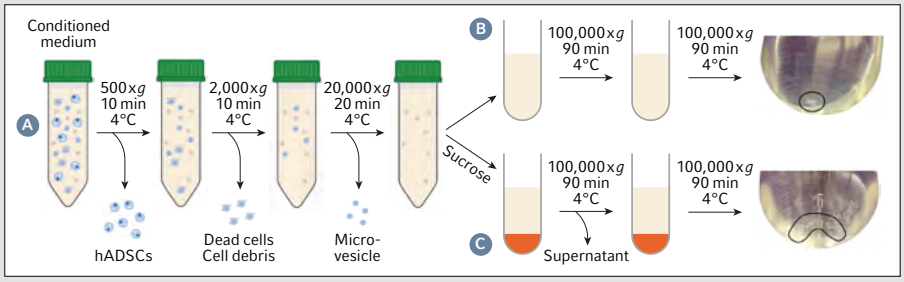


Fig.2: Combination of the Centrifuge CR22N and Rotor R15A to clear the media and ultracentrifugation without sucrose cushion or with sucrose cushion to pellet the exosomes using the Ultracentrifuge CP100NX and Rotor P32ST. Created with www.biorender.com

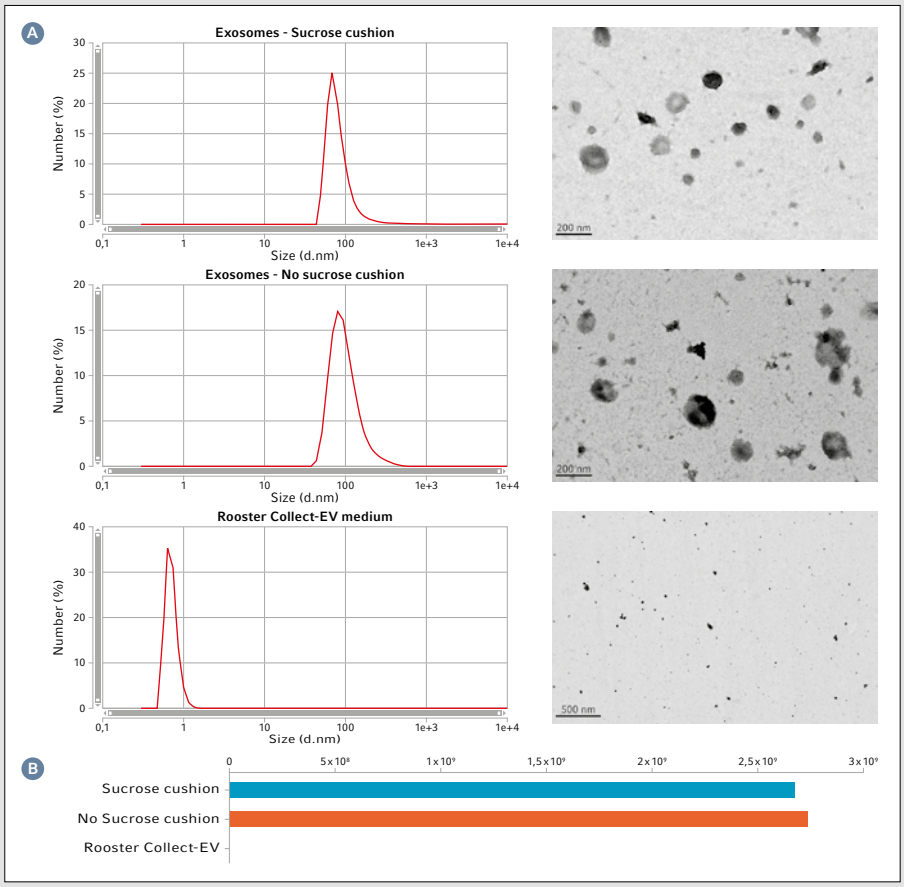


Fig.3: (A) Dynamic Light Scattering analysis (left) and electron microscopy (right) of exosomes without or with sucrose cushion. (B) ELISA quantification from the same exosomes samples

Exosome characterization and quantification

DLS measurements of exosomes isolated without or with sucrose cushion showed both peaks around 100 nm, confirming the presence of only exosomes in the PBS (Fig. 3, A).

Peak width of exosomes without sucrose cushion is bigger and tends to shift to the higher size detection. In comparison, the peak width of exosomes with sucrose cushion is relatively smaller and concentrated around the lower size. Therefore, the exosome population with sucrose cushion is more homogeneous and intact. This data was confirmed by using the electron microscopy technique (Fig. 3, A). The population of exosomes without sucrose cushion was bigger and heterogeneous, while the exosomes in the sucrose cushion were smaller and homogeneous with less debris detected. The expression of exosomal biomarkers (CD63) was analyzed by ELISA (Fig. 3, B).

The high relative content of CD63 was found in the same quantity in both exosome isolation techniques. These data confirm that exosomes can be isolated using the Centrifuge CR22N and Ultracentrifuge CP100NX and that the one-step sucrose cushion technique allows the collecting of a rich and homogeneous population of exosomes by reducing the *g*-force (stress) applied to them compared to no sucrose cushion technique.

Conclusion

As demonstrated by the present work, the DASbox Mini Bioreactor System equipped with BioBLU 0.3 Single-Use Bioreactors coupled with the Centrifuge CR22N and the Ultracentrifuge CP100NX is a great combination for the successful collection of an intact and homogeneous population of exosomes derived from hADSCs with a sucrose cushion.

Download of full **Application Note 476**

BERRIT HOFF, EPPENDORF SE

No Wish Unfulfilled When It Comes To Centrifugation

With the acquisition of the renowned centrifuge business of Japanese Koki Holdings Co., Ltd., in 2020, Eppendorf was able to complete its centrifuge portfolio. Under the motto of our campaign "Start Separation at Ease", we are offering you comprehensive solutions for every separation task. Learn more in this interview with Global Marketing Manager Dr. Marc-Manuel Hahn.

Comprehensive solutions for every separation task

BioNews: Marc, the centrifuge team at Eppendorf has gone through a very exciting period over the past years. How did you perceive this phase?

Marc-Manuel Hahn: This period of time was very dynamic and intense for all of us. I was especially impressed by how quickly the Japanese colleagues at Eppendorf Himac Technologies became a part of the Eppendorf family.

BN: What inspired you in particular?

MMH: Without a doubt – the new team's vast expertise! But then again, this is not surprising as this subsidiary company has been specializing in the development and production of ultracentrifuges since 1955, which was later followed by floor-standing centrifuges and high-speed centrifuges. With high-end instruments such as these, innovative safety features such as contactless imbalance detection and Rotor Life Management play a large role. Extensive expertise is crucial and indispensable.

BN: What is the position of Eppendorf as a supplier of centrifuges today?

MMH: As a premium supplier of separation technologies, we are now offering one-stop shopping – from microcentrifuges and benchtop centrifuges all the way to high-speed centrifuges and ultracentrifuges. In this way, we can provide our customers with the right solution for their applications. Here, our increasingly sustainable solutions extend beyond the centrifuge: in addition to rotors and adapters, they include consumables as well as digital products and customized services. In summary: we offer everything that is needed to achieve the best performance and the best results every day – reliably and reproducibly.

BN: Is this also the idea behind the "Start Separation at Ease" campaign?

MMH: Exactly! We want to make the daily centrifugation routine as easy as possible for researchers so that they may take care of the essentials – their research. This is why we continually work on understanding their challenges and problems in the laboratory as well as predict trends wherever possible. It is only in this way that we can offer users customized solutions for their needs – today and in the future.

www.eppendorf.com/your-centrifuge-solution

HANAË KÖNIG, EPPENDORF SE

PCR: Need for Speed?

After its invention in 1985, PCR started its triumphal procession in the early 1990s. At that time, a PCR cycle took 4–5 hours, meaning that half a working day had to be spent holding a tube and moving it from water bath to water bath, while counting seconds and trying to maintain the right temperature.

Enhancing speed has been one key objective for cycler manufacturers since then. Fortunately, the components needed for the heat transfer in a PCR instrument became better and better in quality and performance, i.e. Peltier elements and thermoblock materials.

Taking advantage of major technological advances over the years, Eppendorf succeeded in developing a PCR cycler which is probably the fastest model on the market: Mastercycler® X50s. It features a silver block for fast heat transfer and Peltier elements of excellent quality enabling PCR runs with heating rates of 10 °C/s. Its innovative 2D-Gradient technology allows testing of 96 different combinations of denaturation and annealing temperatures in one run in order to optimize yield and specificity of a PCR. This does not speed up the run itself, but the process to determine the optimal conditions for the DNA and primer pair takes less time.

PCR kit manufacturers, too, dedicate their expertise to enhancing the speed of PCR. Their aim is to find more reliable, stable, and fast enzymes for PCR preparation. Thanks to new technologies and kits, the runtime of a PCR can nowadays be reduced from 1 hour down to 15 minutes while keeping the same quality of results.

Make the most of your working day and spend the time you win with your PCR results and new findings! Speed up your PCR with Mastercycler X50s + Fast PCR enzymes, e.g. from Solis BioDyne.

Learn more in our online articles:

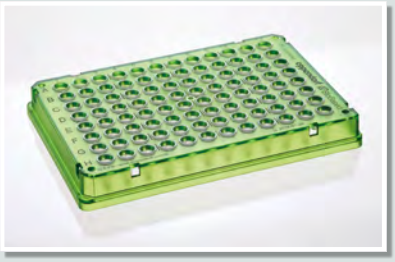
> "Multiple targets, one run: multiplex your PCR!"

> "Higher faster further"

Tip

More Sustainable PCR Plates

Plastic consumables are indispensable in many laboratories. These consumables based on fossil raw materials constitute a considerable challenge with respect to sustainability.



Already in 2022, we started using recycled and renewable feedstocks in our products. This 2nd generation renewable feedstock is based on biobased waste and residues. The renewable feedstocks used to produce the plastic raw materials can be traced back to the first collection points, and the origin of the renewable raw materials from carefully selected suppliers is documented. The final polymers are sustainability certified by "ISCC PLUS" – a leading global certification scheme for biobased polymers.

After launching Eppendorf Tubes® BioBased and epT.I.P.S.® BioBased pipette tips, we now also offer the popular Eppendorf twin.tec® PCR plates, skirted, as a "BioBased" variant. Both materials, the polycarbonate of the frame as well as the polypropylene of the wells are made from renewable feedstock.

Your benefit: a PCR plate with 100 % identical technical performance and a share of at least 86 % renewable polymers generated with electricity from 100 % renewable energies (hydropower).

Improve your lab's carbon footprint – with Eppendorf BioBased consumables.

www.eppendorf.com/biobased

BARBRO PATTERSON, EPPENDORF SE

PCR: Zero Tolerance for Deviations

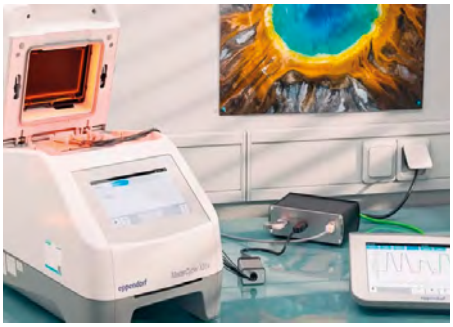
Many factors influence PCR: setup, reagents, reaction vessels, environmental changes, and the technical parameters of a PCR cycler. The performance of the cycler's thermoblock plays a major role in obtaining accurate and reproducible results. In fact, the temperature of the thermoblock is critical for a successful PCR.

Exact adherence to defined temperatures is a prerequisite for reproducible PCR results. Deviations can lead to impaired denaturation efficiency, mispriming in the annealing phase, or compromised polymerase activity during elongation. An inaccurate or inhomogeneous temperature profile can manifest itself in artificial PCR products, low or no product, or varying results within one PCR plate. This can happen when the Peltier elements responsible for heating and cooling do not perform properly.

A common obstacle is that PCR cyclers from different manufacturers may differ when it comes to temperature control making it difficult to achieve comparable PCR results.

Good reasons for temperature verification

Are you concerned about reliable and reproducible results? Do you want to maintain your cycler's quality and reliability for a long service life? Do you want to make sure that environmental changes are not affecting the quality of your results? Or do you work in a regulated environment such as pharma, in which preventive maintenance with instrument qualification is part of your daily quality control? These are all good reasons for temperature verification.



Also, when you use different cyclers for the same protocol, a comparison of temperature verification results from different cyclers gives you clarity before a PCR run – or may explain differences in the results.

Do it yourself or let us do the job

Do-it-yourself temperature verification is no problem with the right equipment, but there are several important details to consider.

Why not reduce your administrative and operative workload and let us do the job? Our qualified full service includes temperature verification and the qualification of other relevant instrument parameters for complete system performance verification.

Learn more in our online article about temperature verification!

Tip

Eppendorf Lab Channel: Webinars and More

Eppendorf Lab Channel is our virtual event platform where you can watch a multitude of webinars – live and on demand at a time of your choice, as individual events or multi-part webinar series. From practical tips and tricks for mastering your daily lab life to talks on cell culture, PCR, liquid handling, centrifugation, bioprocessing as well as digitalization and sustainability – you can look forward to a broad range of topics.

What makes our Lab Channel special is that, in our live sessions, you can engage directly with Eppendorf experts. Pose your questions, and our experts will gladly share their expertise and knowledge with you.

A special highlight awaits you in April 2024 where we will surprise our visitors again with an attractive exhibition stand including auditorium. Mark your calendars for the **analytica trade fair in Munich, Germany, from April 9 to 12!** And don't worry if you can't make it in person; many of the exciting talks will be streamed via Eppendorf Lab Channel.

Curious to learn more?
Then register for free at
eppendorf.link/labchannel

PS: Learn more about events with Eppendorf participation on page 5.

BRIGITTE KLOSE, EPPENDORF SE

Research Keeps Evolving – And Eppi® Keeps Up

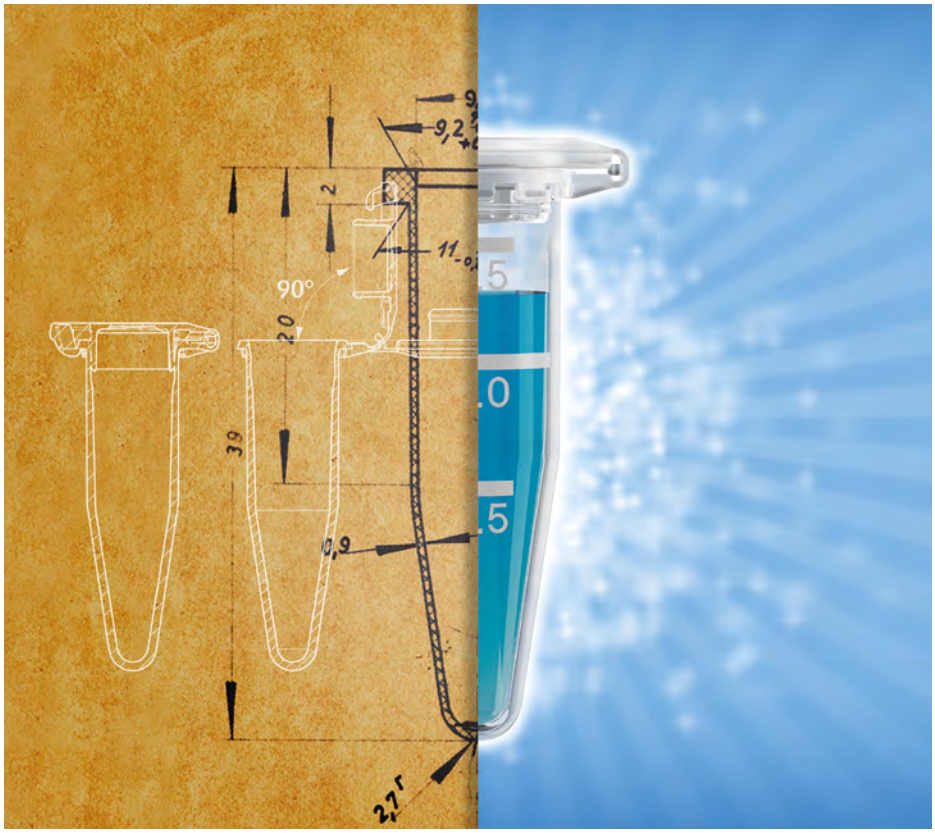
1963 saw the invention of the lava lamp, the cassette tape, and the push-button telephone. One very special invention, however, was the Eppendorf Tube – affectionately called "Eppi". This first single-use microcentrifuge tube, made from polypropylene, revolutionized scientific experiments and processes in the laboratory from the ground up. Read on to learn about the innovation leaps with which Eppi, and subsequent Eppendorf Tubes®, have convinced scientists over the past six decades.

Sold more than 1 billion times, the Eppi is not only the best-known product worldwide which is associated with the Eppendorf name; its name, and the classic silhouette, have in fact become the synonym for microcentrifuge vessels with a volume of 1.5 mL. "Please hand me an Eppi!" or "Where is my Eppi?" – these are statements that are heard every day in laboratories all over the world. But for now, back to the early beginnings …

1960–1980:
Eppendorf Tubes conquer the laboratory

The invention of the first piston-stroke pipette by Eppendorf in 1961 had allowed researchers to dispense liquids in the microliter range safely and accurately. The only thing that was still missing was a suitable sample tube! And so, the "Eppendorf Tube 3810" (its official product name), introduced in 1963, was practically revolutionary, as suddenly, only small volumes of both the expensive reagents as well as the valuable samples were needed. The foundation had been laid for significant advances in the areas of molecular biology and medicine; the Eppi became the industry standard.

With the increasing popularity of the Eppi rose the demand for additional compatible instruments and consumables. Thus, Eppendorf developed the microliter system in 1964 – a complete solution including mixer and centrifuge – with Eppi as the key consumable.



Consistently refined and always adapted to current customer needs, these products comprise the core elements of laboratory work and the Eppendorf product portfolio to this day – with a broad variety of pipettes and pipette tips, centrifuges, mixers, and Eppendorf Tubes for the processing of sample volumes from 0.2 mL to 50 mL.

1980–2010:
Eppi broadens its base

1988: *Eppendorf Safe-Lock Tubes*

The newly developed Safe-Lock snap lid prevents unintended sudden opening, and therefore sample loss, during centrifugation, incubation, and storage.

Safe-Lock tubes offer high safety, e. g. when handling toxic substances.

1992: *Biopur®*

All tubes available in the Biopur purity grade are certified sterile, pyrogen-free, RNase-free, DNase-free, DNA-free, and ATP-free as well as free of PCR inhibitors. This is achieved through a complex automated production process, the levels of control of which preclude any kind of contamination with biological substances.

2004: *DNA LoBind® and Protein LoBind® Tubes*

The specialized Eppendorf LoBind material without surface coating guarantees maximum recovery of DNA and RNA molecules or excellent recovery of proteins, respectively. For improved experimental results in sophisticated applications.

2005: *g-Safe® – centrifugation stability up to 30,000 x g*

The Eppendorf palette of powerful microcentrifuges is growing, enabling faster, more effective centrifugation runs. In order to keep up with these innovations and prevent sample loss through tube breakage, Eppendorf Tubes are optimized to the exceptional centrifugation stability of up to 30,000 x *g*.

2010 until today:
Smart and sustainable innovations

2013: *Eppendorf Tubes 5.0 mL*

The 5.0 mL tube (available with snap cap or screw cap) closes the large gap between the 1.5 mL Eppi and 15 mL conical tubes. They accommodate the processing of larger volumes than the standard Eppi while allowing improved sample access as compared to the long 15 mL tube. The 5.0 mL system includes complete accessories for centrifugation, heating/mixing, and sample storage.

2019: *Eppendorf Conical Tubes 25 mL*

The Eppendorf Conical Tube 25 mL takes its place between the conventional conical vessels of the 15 mL and 50 mL volumes. While it features the same diameter as the conical tube 50 mL, it is not as long. The reduced immersion depth of the pipette into the vessel thus minimizes contamination risk.

Eppendorf Conical Tubes 25 mL: lower height, better sample accessibility

The tube is available with either screw cap or the patented* SnapTec® lid.

2022: *Eppendorf Tubes BioBased*

The Eppendorf Tubes BioBased of the sizes 5.0 mL, 15 mL, 25 mL, and 50 mL open up new possibilities of carrying out laboratory work in a considerably more sustainable fashion without endangering experimental results. Through the use of a biobased polymer of the second generation, which is manufactured from min. 90 % renewable raw materials, the product-related CO_2 footprint of these vessels was significantly reduced.

Eppendorf Tubes BioBased make your laboratory work significantly more sustainable

Eppi will stay on top of things – promise!

It is important to Eppendorf to find innovative solutions for the ever-changing needs in the laboratory. We will continue to offer our users novel product variants so that we may improve their daily laboratory experience even more.

More information at
www.eppendorf.com/tubes

*US Patent 8,540,948

News

"Vessels Must Be Labeled"

Naturally, every lab member agrees that vessels must be labeled. In reality you often find vessels in your laboratory without any labeling or with non-readable labeling. Clear labeling is recommended to make reading as easy and as reliable as possible for everyone. Printed labels on vessels in plain writing are the bare minimum for reliable reading. Barcodes or 2D datamatrix codes are one step further towards a fast and safe sample identification.

New! SafeCode barcoded plates

> Pre-labeled barcoded plates with 3-level-coding in different formats enable you to improve your processes.

> Benefit from digitalization at Eppendorf. Download all relevant, ID-specific documentation for your plate, like certificates, drawings, lot numbers, and supplier order numbers using the Eppendorf DataPort.

> For even more convenience: manage your barcoded vessel and your sample with sample management software like eLabNext.

Your benefit

The Eppendorf SafeCode system enables smart labeling of your high-value samples to ensure safe identification and ultimately safe results.

Download brochure!

CORDULA RICHTER AND CAROLYN TAUBERT, EPPENDORF SE

Eppendorf Research Prize Winners Visit Hamburg

Maurice Michel and Ann Kennedy

Last year, we continued the tradition of welcoming the winners of the two Eppendorf research prizes to Hamburg. Ann Kennedy, Ph.D., USA (*Eppendorf und Science Prize for Neurobiology* 2022) and Maurice Michel, Ph.D., Sweden (*Eppendorf Award for Young European Investigators* 2023) were guests at the headquarters of the Eppendorf Group in Hamburg. As is customary on this occasion, the two award winners gave insights into their fields of research through presentations. They themselves learned about the history of the company and the people at Eppendorf. They also got an impression of how our products are manufactured not only at Eppendorf headquarters, but also at our production site for laboratory consumables in Oldenburg.

The farewell gift – a pipette engraved with their name – will remind them of their visit to Hamburg when they are working in the lab in the future.

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

Marissa Scavuzzo

Marissa Scavuzzo wins *Eppendorf & Science Prize* 2023

Congratulations to Marissa Scavuzzo, Ph.D., Postdoctoral Fellow at Case Western Reserve University School of Medicine, Cleveland, USA on winning the 2023 *Eppendorf & Science Prize for Neurobiology*.

The ability to digest food, absorb nutrients, and process waste is required for life. Many of these essential tasks are controlled by an independent nervous system embedded within every layer of the gut called the enteric nervous system. Dr. Scavuzzo studies this network of nervous system cells inside the gastrointestinal tract, often referred to as “the second brain”. She uses stem cells and tissues to create lab-grown models of the mouse and human intestines. By combining these organs-in-a-dish with animal models, she works to map glial cells’ diversity in the gut. Glial cells, the support cells of the brain, aid in regulating and protecting neurons. However, their role in the gut is not well understood. Dr. Scavuzzo aims to understand how enteric glia function in a normal gut and how they react to changes in environment, genetic makeup, or diet. Millions of individuals who suffer from gastrointestinal diseases could be impacted by this work and its potential for creating new and effective therapies.

Win a Set of 3 Pipettes

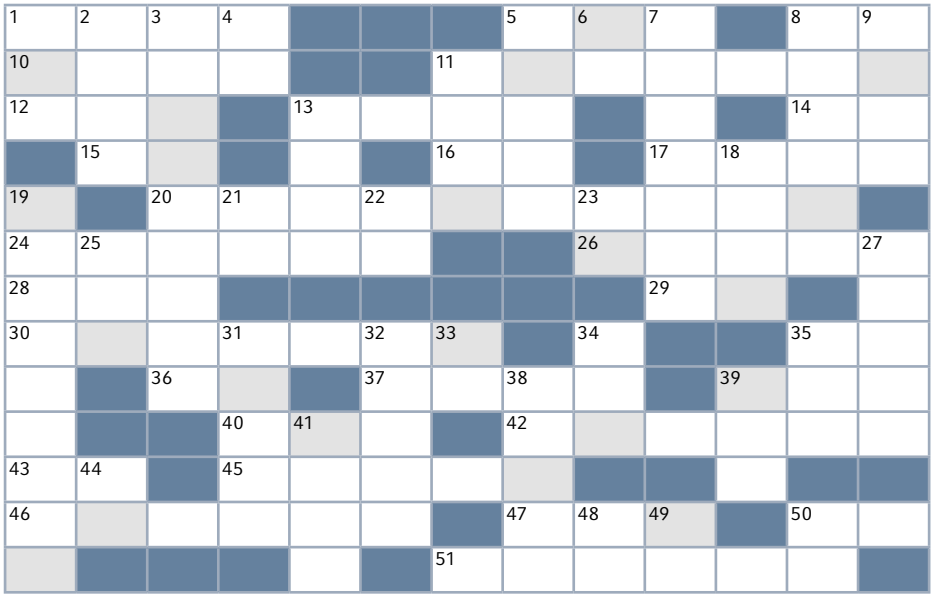
The solution of the prize competition of BioNews No. 58 was "YOUR WORK MATTERS". The main prize, an Eppendorf Xplorer® plus 8-channel pipette, went to Isabelle L., France.

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, 2024**.

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. 62.



1st Prize:

1 Eppendorf Research® plus 3-pack 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 Part of the name of Eppendorf PCR plates
5 Parts per million (abbrev.)
8 Whether San Diego or South Dakota, keep it short
10 Direction
11 Seminar on the www
12 Name part of an early form of modern human in Europe
13 The capital is Muscat
14 Radioactive alkaline earth metal (chem. symbol)
15 0.001 litres (abbrev.)
16 Type of enzyme used by viruses (abbrev.)
17 Air pollution caused by emissions
20 Helps reduce human intervention
24 Giving up is not an …
26 Loved by Elizabeth II since childhood (sing.)
28 This + Tai = legendary
29 Noble gas often used in signs (chem. symbol)
30 Phrase, cliché, platitude
35 Used in solar cells (chem. symbol)
36 Chemical symbol for sodium
37 Migraine phenomenon
39 Fought against by a popular band from Seattle
40 Revolutions per minute (abbrev.)
42 Skilled performer
43 Not out
45 Hopefully secure!
46 Programming language
47 Influential key on computer keyboard
50 *ante meridiem* (abbrev.)
51 Mischievous tricks, jokes

DOWN

1 Same question as 1 across
2 Not cold
3 State of separation
4 Part of the Christian bible (abbrev.)
5 Combining form meaning five (Greek)
6 Between thallium and bismuth (chem. symbol)
7 Repeatedly impossible
8 Long strip of cloth wrapped around the body
9 Glamorous type of queen
13 Engine named after his inventor
18 Not less
19 Consumables used with manual dispensers
21 Where interactions between humans and machines occur (abbrev.)
22 Preposition
23 Chemical element with atomic number 43 (abbrev.)
25 … excellence
27 A novel hero of Dostoevsky
31 The second month of the year to have 31 days
32 American actor and screenwriter (family name)
33 United in diversity (abbrev.)
34 Make love, not …
35 Save our souls
38 Detection system using radio waves
39 Helsinki is the capital (vehicle registration code)
41 Story of a book, film, play
44 If I can make it there, I'll make it anywhere … (abbrev.)
48 Hollywood is one of its neighborhoods (abbrev.)
49 Nashville is the capital (abbrev.)
50 Has the atomic number 33 (chem. symbol)

Solution hint for prize competition of BioNews No. 60:

	P					O							E					E	

Send us the solution until **June 30, 2024**. 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**