BIO NEWS
No. 47 – 2017

eppendorf

Bioprocess Control Made Easy: The New BioFlo® 120

> Smart. Connected. Nize. VisioNize® World Premiere
> New Freezers Protect What Matters – Your Sample
> ep*Motion*® 10 µL System: Improved Pipetting of Sub-Microliter Volumes

Application Notes

How Pipette Tips Influence Results (Part 4) · High Density *E. coli* Fermentation and Protein Production using the Eppendorf BioFlo® 120 Bioprocess Control Station · etc.

Dear Readers,

As one of the leading bioprocess suppliers, we know: Nothing is as constant as change in a bioprocess laboratory! New experimental set-ups are developed, other production organisms are tested, and new personnel must be trained. With the new bioprocess controller BioFlo® 120, we provide you with a user-friendly, flexible system, with which you will conquer these challenges effortlessly (pages 4–5 and Application Notes 3–4).

The trade fair "Labvolution® 2017" in Hanover, Germany, was the setting for the world premiere of VisioNize®, a new system that will allow you to network and efficiently manage manifold Eppendorf instruments. An innovative and inspiring project that was received with enthusiasm by our visitors (page 6).

In addition, we would like to inspire you with the continual development of our customer communication – via more and more channels. On page 12, you will learn more about selected novelties and highlights in our ever-growing information universe.

Sample preparation, sample storage, sample control … the life sciences are all about the sample. For the secure long-term storage of your valuable samples, we especially recommend the new freezers of the CryoCube® F740 series. Higher capacity, optimal energy consumption, and models featuring the VisioNize interface leave nothing to be desired (page 7).

With the new ep*Motion*® 10 µL system for precise pipetting in the sub-microliter range, you are now able to miniaturize sample volumes, for example during the preparation of NGS libraries, thus helping you to achieve considerable savings (page 11). Additional novelties include the microinjectors CellTram® 4r Air and CellTram 4r Oil. They cover a broad range of research applications and allow optimal sample control (page 8).

We hope you will enjoy this issue!

Your BioNews-Team

Imprint

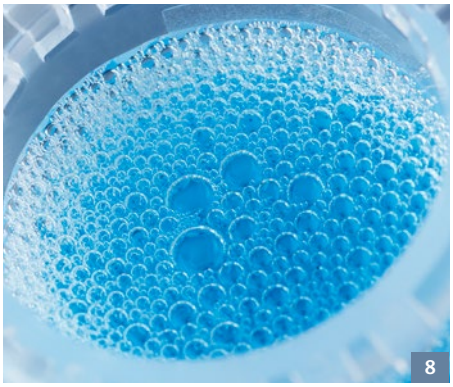
Editorial team
Berrit Hoff (Editor-in-Chief), Axel Jahns, Jochen Müller-Ibeler, Tanja Musiol, Natascha Weiß

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

We welcome all readers' articles for this publication. However, no responsibility is accepted for unsolicited manuscripts.

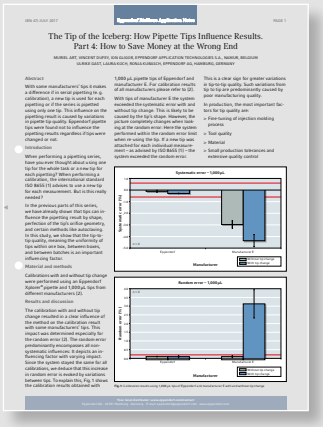
Important note
The new products described may be launched at different times in various countries. Please contact your local Eppendorf organization or distributor for details.

Technical specifications subject to change. Errors and omissions excepted.



IN THE SPOTLIGHT	The New BioFlo® 120 – Bioprocess Control Made Easy	4–5
STRAIGHT FROM THE LAB	Protect What Matters – Your Sample	7
	Better Safe Than Sorry: Verification of Spectrophotometers	9
	Proper Handling of Cells in the Lab (III)	10
	Do Freezing and Cold Storage Impact Sample Quality?	13
INNOVATION	Smart. Connected. Nize. VisioNize®	6
	New Microinjectors for Optimal Sample Control	8
	ep*Motion*® 10 µL System: Improved Pipetting of Sub-Microliter Volumes	11
CLOSE-UP	Caution, Foam!	8
NEWS/TIPS	Bioprocess Performance Plans	5
	Digital Sample Data Collection and Management	6
	A Video Journey Through the Laboratory	10
	Eppendorf Prize Winners 2016/2017: Gilad Evrony & Tom Baden	14
SERVICE	Stay Informed – On All Channels	12
	Trademark Information	14
	Win a Pipette 3-Pack!	15

Eppendorf BioNews Application Notes


The Tip of the Iceberg: How Pipette Tips Influence Results.
Part 4: How to Save Money at the Wrong End

MURIEL ART, VINCENT DUFEY, ION GLIGOR, ULRIKE GAST, LAURA KOCH, RONJA KUBASCH The Tip of the Iceberg: How Pipette Tips Influence Results. Part 4: How to Save Money at the Wrong End	1–2
BIN LI, MA SHA High Density *Escherichia coli* Fermentation and Protein Production using the Eppendorf BioFlo® 120 Bioprocess Control Station	3–4
INES HARTMANN, AURÉLIE TACHENY Superior Well-to-Well Consistency with Eppendorf Cell Culture Plates	5–6
MICHAEL DREIFKE, MICHAEL FRÖBA, MARTIN ARMBRECHT Eppendorf BioSpectrometer® kinetic: Activity Measurements of Enzymes Immobilized on a Silica Matrix	7–8

ULRIKE BECKEN, EPPENDORF AG BIOPROCESS CENTER, JUELICH, GERMANY

The New BioFlo® 120 – Bioprocess Control Made Easy

Learning to use a bioprocess controller is a complex endeavor for the beginner, and even bioprocess specialists need time to get used to new equipment. With the BioFlo 120 we developed a benchtop bioprocess system that is straightforward to set up and easy to use. By reducing the time required in learning to use new equipment, we free up scientists to spend more time on what is important, their research.

Unlimited applications

Work in a bioprocess lab is ever-changing. Experimental set-ups develop, other production organisms are tested, and new users have to be trained. Responding to changing needs becomes much easier with a bioprocess controller that is user-friendly and flexible.

With the BioFlo 120 (Fig. 1) you can grow just about any cell type you can think of – be it microbial, insect, fungal, mammalian, or stem cells – on a single platform. It can be employed for batch, fed-batch, and continuous cultures. This is how you keep your options open without the need for investing in new equipment.

Fig. 1: BioFlo 120 bioprocess control station

Auto Culture modes get you started right away

In bioprocessing, environmental parameters like temperature, pH, and dissolved oxygen (DO) have to be monitored and controlled. Especially the less experienced user might seek guidance: "Which setpoints should I choose, and how should I set up control loops to maintain the actual process values close to them?" Well, that depends, and it can become very complex.

The user has to take into account which organism he is going to cultivate, in what kind of vessel, with how many impellers, and so on, and so on. This is where our newly developed Auto Culture modes come in. We built a control software that is equipped with tried and tested setpoints and control loops for different applications and vessel types.

With the Auto Culture modes of the BioFlo 120 bioprocess control station, the user can select either a pre-defined *E. coli* batch fermentation protocol or a CHO batch cell culture process, which begins at the push of a button (Fig. 2).

The Auto Culture modes are populated with setpoints and cascades recommended by our applications development team, backed by the expertise developed over hundreds of experiments in our applications R&D lab. The user only needs to select the vessel size and type from the list of available choices, and make standard preparations (sensor and pump calibration, vessel preparation) for the run. The Auto Culture modes allow users to achieve quick and easy initial culture success with a minimal learning curve. All setpoints and modes of operation can be adjusted, optimized, and saved as user-defined recipes, which are collected into the Auto Culture library for future use.

Fig. 2: BioFlo 120 Auto Culture mode interface

We tested the Auto Culture mode for *E. coli* in batch fermentation of a GFP-expressing strain. As shown in Fig. 3, within six hours, the OD_{600} value reached 14 and the GFP production was 650 relative fluorescence units/mL. The growth curve is typical for a batch fermentation and provides necessary strain characterization information to begin designing a fed-batch or continuous bioprocess.

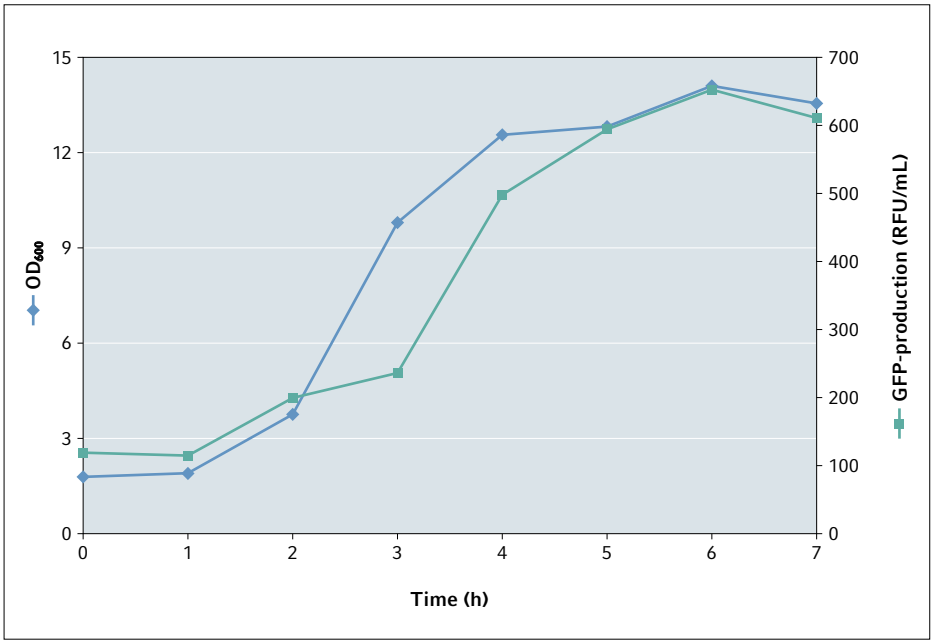


Fig. 3: *E. coli* growth curve and GFP production yield. RFU: Relative fluorescence units

The Auto Culture modes are integrated into the embedded bioprocess control software. The software offers real-time local process control through an integrated touchscreen. For additional process control capabilities and secure database management the BioFlo 120 can also be connected to Eppendorf SCADA platforms DASware® and BioCommand®.

Scalable solution

Today you perform initial tests in a small working volume, but tomorrow you might want to scale up your bioprocess to obtain more of the desired product.

The BioFlo 120 makes it possible. It is equipped for use with BioBLU® Single-Use Vessels as well as industry-standard glass autoclavable vessels. Together the vessels cover a working volume range of 250 mL to 40 L.

In case you previously used a BioFlo 110 or BioFlo/CelliGen® 115 bioprocess controller, keep your vessels! We offer conversion kits to easily use them with the new BioFlo 120 control station.

Feature-packed

With the option of mass flow-controlled gassing and automatic mixing of up to four gasses, the control station is well equipped for DO control in a variety of applications.

User-defined DO cascades offer additional process flexibility. Universal connections for digital Mettler Toledo® ISM® and analog sensors make it easy to monitor a variety of critical process parameters. Additionally, we minimized the footprint of the bioprocess control station to save precious lab space.

Performance meets value

The Eppendorf BioFlo 120 offers simplicity and ease of use, without sacrificing capability. No matter if you are in an academic, governmental, or industrial research setting; working with bacteria, yeasts, fungi, mammalian, insect or plant cells, the BioFlo 120 fermentor/bioreactor provides solutions for what you need today, and for what you will encounter in the future.

News

Bioprocess Performance Plans

Technologically demanding products require first-class service to ensure that the results they produce are optimized. Customers can rely on Eppendorf Service for superior support for their bioprocess products, beginning with startup of the system. The services range from technical support and troubleshooting to delivery of replacement parts on short notice and customer-specific maintenance programs.

Preventive Maintenance

As with all complex technical systems, Eppendorf bioprocess equipment should be maintained regularly to keep all parts in good working order. This maintenance avoids cost-intensive down times and contributes to preservation of value. We recommend complete preventive maintenance once a year. Additionally, we encourage users to execute certain maintenance actions prior to every run or in regular cycles (e.g. every month). We will be happy to advise.

epServices
for premium performance

Performance Plans feature:

> A choice of preventive maintenance programs covering inspection and maintenance work
> Validation and adjustment of operating parameters in accordance to Eppendorf specifications
> Installation Qualification and Operational Qualification certification
> Full documentation

Your benefits:

> Ensure reliable operation
> Avoid expensive repairs
> Prevent prolonged downtime

For more information please visit
www.eppendorf.com/epServices*

*Performance Plans (also for other Eppendorf products) are available in selected countries only.

TANJA MUSIOL, EPPENDORF AG

Smart. Connected. Nize.

The Labvolution® 2017 in Hanover, Germany, was once again entirely dedicated to the laboratory of the future. In addition to the special exhibition area smartLAB, where different cooperation partners presented a future vision of the laboratory, an attractive framework program offered seminars and lectures in and around the topic areas of workflow optimization, digitalization, and emerging technologies.

With its exhibition booth as well as its visitor program, Eppendorf focused entirely on the theme of the exhibition. During tours of the "Glass Laboratory", visitors learned everything about the optimization of work steps in a cell culture laboratory. The Eppendorf Cell Handling experts offered practical tips and tricks on proper handling of cells in the lab – with special emphasis on the prevention of contamination. Eppendorf offered a seminar on this very topic, during which the questions of visitors were addressed in detail. Visitors were also invited to learn more about new developments in the fields of digitalization and emerging technologies. Once again this year, Eppendorf contributed to the set-up of the smartLAB – with products, lectures, and a podium discussion intended to illuminate all aspects of the laboratory of the future.

A workshop during which users had the opportunity to discuss their ideas and wishes with respect to the laboratory of the future was a further highlight.

VisioNize® world premiere

The Eppendorf booth itself was fully connected. At the world premiere of VisioNize, the visitors learned how soon they will be able to connect various Eppendorf instruments and thus monitor and manage them in a smart network. The central software application allows the user to view the current status of the connected equipment from practically anywhere. For example, the temperature of a freezer (also see page 7) can be displayed and recorded for documentation and analysis purposes. In case threshold settings are exceeded, the user receives an e-mail notification from the VisioNize system.

Successful world premiere at the Labvolution exhibition: VisioNize

The visitors agreed: With VisioNize, a part of the future has become reality today.

VisioNize

www.eppendorf.com/visionize

News

Digital Sample Data Collection and Management

The company Bio-ITech, part of the Eppendorf group since Spring 2017, (www.bio-itech.com) complements the creation of lab equipment networks by offering software solutions for digital management and documentation of experiment results and sample data. For example, the laboratory inventory system eLABInventory supports the organization and management of samples, e.g. in freezers with the help of barcodes.

The sample is thus easily identified within the software via different search options. Furthermore, if the eLABJournal®, an electronic lab notebook, is used, samples can be linked to their respective experimental data and SOPs. Thus, not only information on the identity of a sample, but also its experiment history is at your fingertips.

Digital collection and management of sample information and experiment data is steadily gaining in significance in today's environment. In connection with the collection of relevant instrument data, e.g. with the help of the VisioNize system by Eppendorf, the entire history of a sample may be stored and analyzed. All parameters taken together will allow qualitative evaluation of a sample, even after longer storage periods or after passing various workflow steps.

JAN-HENDRIK BEBERMEIER, EPPENDORF AG

Protect What Matters – Your Sample

The life sciences revolve around the sample: Your processes and workflows require you to invest a lot of time and resources. So much of your success depends on care and handling of your valuable samples. After spending countless hours on the many devices and steps utilized to create the sample, have you adequately considered where you will store it? Does your long-term storage solution live up to the value of your process and sample?

The CryoCube® F740 ULT freezer is the latest addition to the Eppendorf Freezer Family. Combining the high-quality tradition of our previous freezers with an increased capacity, the CryoCube F740 series is designed to securely store more of your samples in up to 576 boxes while maintaining optimal energy usage.

Secure storage of up to 576 boxes

The Eppendorf R&D team had a key goal: Take what was great and make it better, e.g. equip the known and proven Eppendorf freezer body concept with new interface technologies to offer our users enhanced application benefits.

The series is based on 5 models:

> The classic model F740
> The touch screen equipped F740i
> The green freezer (with touch screen) F740hi
> Water-cooled models (with touch screen) F740iw and F740hiw

Integrating the Eppendorf PhysioCare Concept® during development has made the CryoCube F740 easier and more comfortable to use: It reduces user stress through a reduced noise level, supports an ergonomic workspace, and ensures an optimal workflow in the lab.

The classic freezer attributes you can expect are:

> Enhanced sample safety through Vacuum-Insulation-Panel reinforced PU foam insulation
> Proven two-stage compressor cascade systems
> Optimal temperature accuracy for long-term sample safety supported by compartment shelves with airways for better and faster inner temperature adaptation
> Insulated inner doors with gaskets to keep the cold in the compartment
> Built-in flat and flexible outer door gaskets to reduce icing effects
> A choice of 3 or 5 compartments and left or right-opening outer door for flexible freezer configuration

Fit for the future

We also wanted to adapt the freezer family to future-proof interfacing: Alarm notifications are relayed via Ethernet, RS485, or building management system interface (BMS). A big touch screen for easy information access enables temperature monitoring and tracking. These data as well as logged alarms and notifications can easily be exported by USB.

Personalized access control for highest sample safety is provided by PIN if necessary.

Model F740i with touch screen

The “i” versions of the CryoCube F740 can be directly connected by VisioNize®, the new Eppendorf network solution (see page 6).

More information at
www.eppendorf.com/freezers

RUDOLF WALCZAK, EPPENDORF AG

New Microinjectors for Optimal Sample Control

With 30 years of cell manipulation expertise, Eppendorf is your expert partner for micromanipulation and microinjection in the life sciences. As a systems provider for a diverse range of applications, we can support you with a solution for each of your cell manipulation requirements. The new CellTram® 4r Air and CellTram 4r Oil are manual microinjectors suitable for a broad range of life science applications.

NEW! CellTram 4r Air and CellTram 4r Oil cover a wide range of research applications and suit all individual working techniques.

CellTram 4r Air

The CellTram 4r Air is a pneumatic injector for oil-free micromanipulation and gentle holding of cells in suspension. It is highly suitable for the uptake and injection of cells – with a performance comparable to that of oil-filled injectors.

This means you can now set up your workstation with air injectors on both the holding and injection side and conduct microinjections entirely oil-free, without the need for refilling oil and without oil spills in the work space. Additionally, the new coarse and fine drive and the piston position scale allow you to set the injector's pressure characteristics according to your needs.

CellTram 4r Oil

The CellTram 4r Oil is an hydraulic, oil-filled microinjector. It provides more direct sample control and can generate higher pressures than an air system.

CellTram 4r Oil is ideal for sophisticated applications demanding high resolution and sensitivity. It has an improved oil-filling system for minimal interruption of the workflow. A special self-closing valve helps to prevent spillage of oil during the filling process.

Both CellTram 4r models have been designed with special emphasis on excellent ergonomics, ease of use, and high precision. They also feature improved accessories, such as the new scaled Capillary Holder 4 for reproducible mounting, and the new Grip Head 4 System for easier capillary exchange and higher user safety.

Both models can be used with all common micromanipulation systems, yet they develop their full potential with Eppendorf manipulators.

The products described are for research use only.

More information at
www.eppendorf.com/cellmanipulation

Close-Up

Caution, Foam!

Pipettes working with an air-cushion principle are most commonly used in labs around the world and deliver highly accurate results for most pipetting applications. However, the precision and reproducibility of the pipetting results can be affected when working with problem liquids, such as foaming solutions containing high amounts of protein like BSA-solution (bovine serum albumin) or cell culture medium. One method to face this very challenge is reverse pipetting. However, the positive displacement system of Multipette® (U.S./CAN: Repeater®) and Combitips advanced® dispenser tips is even more suitable. This system simplifies the pipetting process by excluding difficulties caused by an air-cushion and secures accurate and reproducible results. Additionally, it eliminates the risk of cross-contamination and thus protects user and instrument.

Foaming solution in an Eppendorf tube. Precise pipetting can only be achieved by using either a special technique or a positive displacement instrument.

Multipette and Combitips also offer considerable handling advantages when pipetting the following problem liquids:

- Viscous liquids with poor flow characteristics, e.g. glycerol
- Liquids of density different from water, e.g. sulfuric acid
- Liquids with high vapor pressure, e.g. acetone
- Hazardous solutions whose aerosols may contaminate the pipette, e.g. phenol
- Infectious liquids with multiple properties differing from water, e.g. blood or serum
- Detergent-containing solutions lowering the surface tension of water, e.g. PCR mastermix

More details at **handling-solutions.eppendorf.com/liquid-handling**

The Tip of the Iceberg: How Pipette Tips Influence Results. Part 4: How to Save Money at the Wrong End

MURIEL ART, VINCENT DUFEY, ION GLIGOR, EPPENDORF APPLICATION TECHNOLOGIES S.A., NAMUR, BELGIUM
ULRIKE GAST, LAURA KOCH, RONJA KUBASCH, EPPENDORF AG, HAMBURG, GERMANY

Abstract

With some manufacturers' tips it makes a difference if in serial pipetting (e.g. calibration), a new tip is used for each pipetting or if the series is pipetted using only one tip. This influence on the pipetting result is caused by variations in pipette tip quality. Eppendorf pipette tips were found not to influence the pipetting results regardless if tips were changed or not.

Introduction

When performing a pipetting series, have you ever thought about using one tip for the whole task or a new tip for each pipetting? When performing a calibration, the international standard ISO 8655 [1] advises to use a new tip for each measurement. But is this really needed?

In the previous parts of this series, we have already shown that tips can influence the pipetting result by shape, perfection of the tip's orifice geometry, and certain methods like autoclaving. In this study, we show that the tip-to-tip quality, meaning the uniformity of tips within one box, between boxes, and between batches is an important influencing factor.

Material and methods

Calibrations with and without tip change were performed using an Eppendorf Xplorer® pipette and 1,000 µL tips from different manufacturers [2].

Results and discussion

The calibration with and without tip change resulted in a clear influence of the method on the calibration result with some manufacturers' tips. This impact was determined especially for the random error [2]. The random error predominantly encompasses all non-systematic influences: It depicts an influencing factor with varying impact. Since the system stayed the same for all calibrations, we deduce that this increase in random error is evoked by variations between tips. To explain this, Fig. 1 shows the calibration results obtained with 1,000 µL pipette tips of Eppendorf and manufacturer E. For calibration results of all manufacturers please refer to [2].

With tips of manufacturer E the system exceeded the systematic error with and without tip change. This is likely to be caused by the tip's shape. However, the picture completely changes when looking at the random error: Here the system performed within the random error limit when re-using the tip. If a new tip was attached for each individual measurement – as advised by ISO 8655 [1] – the system exceeded the random error.

This is a clear sign for greater variations in tip-to-tip quality. Such variations from tip to tip are predominantly caused by poor manufacturing quality.

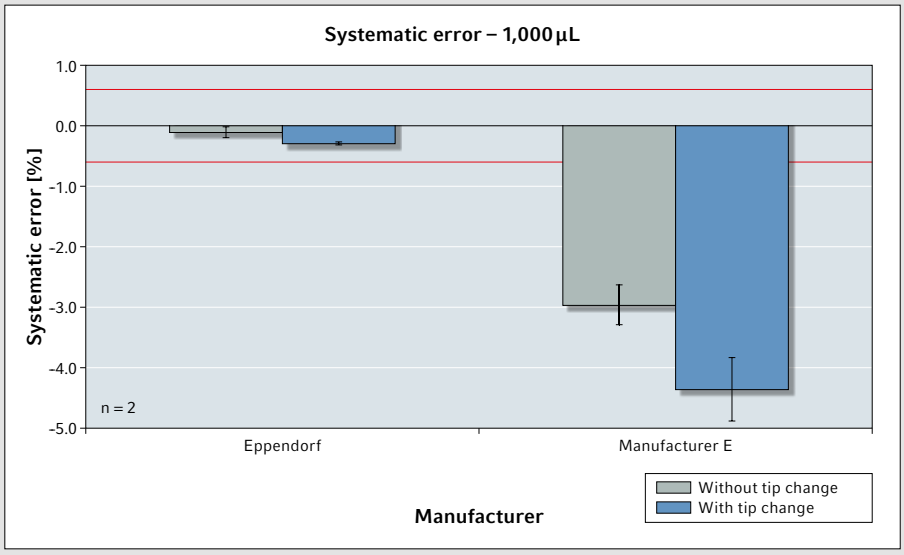
In production, the most important factors for tip quality are:

> Fine-tuning of injection molding process

> Tool quality

> Material

> Small production tolerances and extensive quality control



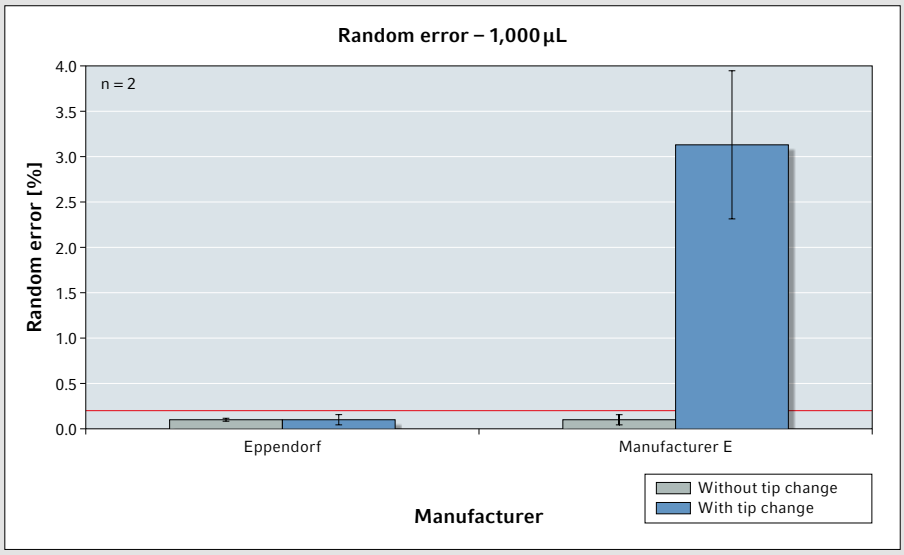


Fig. 1: Calibration results using 1,000 µL tips of Eppendorf and manufacturer E with and without tip change

The Tip of the Iceberg: How Pipette Tips Influence Results. Part 4: How to Save Money at the Wrong End

The tools are the "sacred cores" of any tip production. Their perfection of shape and surface is of significant importance for the tip quality. During tip production, the tools must withstand e.g. a pressure of over 1,000 bar. This means a high load. Consequently, the maintenance cycles of the tools play an important role in product quality. However, the maintenance of tools is a time-, man-power-, and material-consuming process, in short: cost-intensive. Of course, it is possible to use the tools until they are no longer dimensionally stable.

This reduces costs but also reduces the product quality. Hence, the frequency of tool maintenance is driven by the product's compliance with production tolerances. Production tolerances are set by each manufacturer individually and are used to define if the manufactured tips are okay or not.

Eppendorf conducts intensive quality control by short sampling cycles and a high number of tests. Two labs are responsible for the quality testing, a dimensional testing lab and an applicational testing lab. The labs apply narrow error tolerances, leading to Eppendorf's high and reproducible tip quality. Indeed no influence of tip change on the calibration results was discovered in our study [2].

Conclusion

Manufacturers of pipettes and tips, so-called system providers, offer their customers a widely unnoticed additional service: They produce a system instead of single parts. This means, for example, that the production tolerances of the pipette cone are aligned to the production tolerances of the tips. Coordination of the production tolerances is a feature which a non-system provider cannot achieve. Furthermore, based on ISO 8655, system providers have a natural interest to ensure (and certify) that the manufactured system "pipette and tip" performs within the published error tolerances at the date of purchase.

This means that system providers take care that the production tolerances for tips are tight enough to be able to certify the system being within published error limits – regardless of the batch or individual tip.

Manufacturers exclusively producing tips do not have this requirement, thus have the freedom to apply wider production tolerances – to the detriment of the product quality.

The international standard ISO 8655 advises to calibrate pipettes with changing the tip for each measurement. Thus it focuses the performance of the system.

Pipette tips of high quality and homogeneity do not show a relevant influence on the pipetting result.

Only with tip change, variations caused by poor tip-to-tip quality can be detected. It has to be taken into account that the system can be adjusted to a failed systematic error but cannot be adjusted to a failed random error, as caused by poor tip-to-tip quality.

In contrast, a calibration without tip change would only focus on the performance of the pipette. It would only be possible if tips of homogeneous quality were used. With such tips of high homogeneity it would not make a relevant difference if in the daily lab routine a pipetting series is performed with or without tip change.

Within this series we have seen that tips may influence the pipetting result by their shape and the quality of orifice. Also certain methods like autoclaving and tip change can influence the tip and thus the pipetting accuracy and reproducibility. These results are in accordance with the recommendations of the ISO 8655 which describes that pipettes and tips build a system which needs additional calibration if alternative tips are used. In order not to save money at the wrong end, pipette tips should be chosen with the same care as the pipette.

Literature

[1] DIN EN ISO 8655:2002. Piston-operated volumetric apparatus. Beuth-Verlag, Berlin, Germany

[2] Art M, Dufey V, Gast U, Gligor I, Koch L, Kubasch R: Application Note 354: The Tip of the Iceberg: How Pipette Tips Influence Results. www.eppendorf.com/appnote354

High Density *Escherichia coli* Fermentation and Protein Production using the Eppendorf BioFlo® 120 Bioprocess Control Station

BIN LI AND MA SHA, EPPENDORF, INC., ENFIELD, CT, USA
CONTACT: BECKEN.U@EPPENDORF.COM

Abstract

We used the BioFlo 120 bioprocess control station for the fed-batch fermentation of a GFP-expressing *Escherichia coli* (*E. coli*) strain. To quantify cell density and protein production we measured the optical density (OD_{600}) of the culture and the fluorescence of GFP, respectively. After nine hours of culture, the optical density had increased to 191 and the relative fluorescence units (RFU) to 12,532. These results indicate that the BioFlo 120 supports the growth of *E. coli* to high densities and the production of large amounts of protein.

BioFlo® 120 hardware configuration	
Parameter	*Configuration*
Gas Mix	Automatic gas mix
Gas Flow Control	One thermal mass flow controller (TMFC) with 0–20 standard liters per minute (SLPM) flow range
Vessel	2 L heat-blanketed glass vessel with baffle assembly (maximum working volume of 2.2 L)
Motor	Direct drive motor
Impeller	Two Rushton impellers
Sparger	Ring Sparger (Macrosparger)

Table 1: BioFlo 120 hardware configuration

Introduction

The BioFlo 120 (Fig. 1) is a benchtop bioprocess system with the flexibility to control both autoclavable and single-use vessels. The system has proprietary software to monitor and control a wide array of fermentation and cell culture applications, and can be employed for batch, fed-batch, perfusion, and continuous cultures.

The BioFlo 120 supports the use of BioBLU® Single-Use Vessels as well as industry-standard glass autoclavable vessels. With the option of mass flow controlled gassing and automatic mixing of up to four gasses, the control station is well equipped for dissolved oxygen (DO) control in a variety of applications, including high-density mammalian cell culture and bacteria/yeast fermentations.

In the project described in this Application Note, we tested the suitability of the BioFlo 120 bioprocess control station for high-density *E. coli* fermentation.

Materials and methods

Fermentation

We used a GFP-expressing *E. coli* strain (ATCC® 25922GFP™). It was cultivated in a chemically defined medium [1].

Fig. 1: BioFlo 120 bioprocess control station with autoclavable glass vessel

We prepared a pre-culture as described previously [1].

We ran the fermentation in a heat-blanketed glass vessel connected to a BioFlo 120 bioprocess control station. The configurations of the controller and the vessel are outlined in Table 1.

We inoculated the culture with 150 mL of the preculture (10 % of the initial working volume).

The fermentation was carried out at 37 °C. The pH was controlled at 7.0 (± 0.1) and the DO was set to 30 %. Antifoam 204 (Sigma-Aldrich®) was added only when needed.

Culture feeding

Starting 3.5 h after inoculation, the culture was fed with a concentrated feed solution. Pump 2 was assigned as the feeding pump and controlled by the BioCommand® SCADA Software. We set the period of pump control time to 10 s.

Analysis

To monitor the fermentation offline, we took a 3 mL to 5 mL sample hourly using the swabable Luer lock port. We monitored cell growth by measuring the optical density of the culture (OD_{600}) with an Eppendorf BioSpectrometer® kinetic photometer. To measure GFP production we released GFP from the cells to the supernatant using a Bacterial Cell Lysis Kit (GoldBio®, USA) [4] and quantified GFP fluorescence with an Eppendorf BioSpectrometer fluorescence

High Density *Escherichia coli* Fermentation and Protein Production using the Eppendorf BioFlo® 120 Bioprocess Control Station

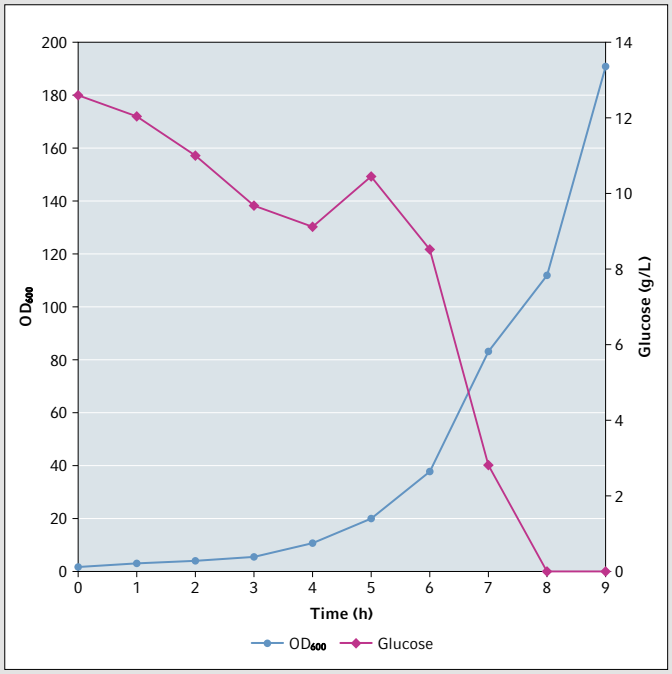


Fig. 3: *E. coli* growth curve and glucose concentration

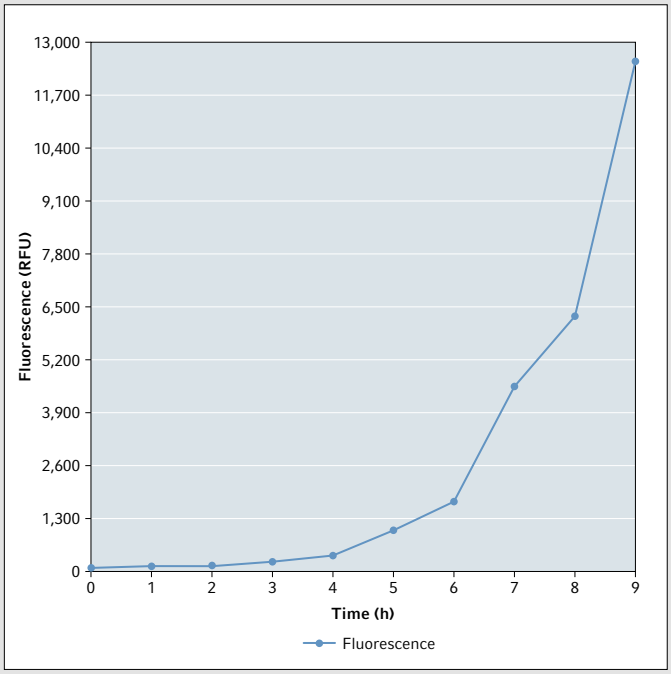


Fig. 4: GFP protein production

photometer. We determined the glucose concentration in the medium using a Stat Profile® Prime Analyzer (Nova Biomedical, USA).

Results

Within 9 h the culture grew to an optical density of almost 200 (Fig. 3). Parallel to the increase of biomass the amount of GFP rose and reached almost 13,000 relative fluorescence units after 9 h. The glucose concentration dropped slowly during the first 4 h, increased with the addition of the glucose-containing feed medium, and then declined rapidly (Fig. 3).

Conclusion

We used the BioFlo 120 bioprocess control station for an *E. coli* fed-batch fermentation. Within 9 h the culture grew to an OD_{600} of 191 and produced almost 13,000 RFU of GFP. These results indicate that with the BioFlo 120 control station and vessels high cell densities can be reached.

Literature

[1] Li B, Sha M. Scale-Up of *Escherichia coli* Fermentation from Small Scale to Pilot Scale Using Eppendorf Fermentation Systems. Eppendorf Application Note No. 306, 2016. www.eppendorf.com/appnote306

[2] Geerlof A. High cell-density fermentation of *Escherichia coli*, 2008. www.helmholtz-muenchen.de/pepf/protocols/expression

[3] Korz DJ, Rinas U, Hellmuth K, Sanders EA, Deckwer WD. Simple fed-batch technique for high cell density cultivation of *Escherichia coli*. Journal of Biotechnology 1995; 39(1):59-65.

[4] Bacterial Cell Lysis Kit Protocol. www.goldbio.com/documents/1358/Bacterial+Cell+Lysis+Buffer-Product+Information+and+Protocol+v1.2.pdf

The complete version of this Application Note 307 can be downloaded at www.eppendorf.com/appnote307.

Superior Well-to-Well Consistency with Eppendorf Cell Culture Plates

INES HARTMANN, EPPENDORF AG, HAMBURG, GERMANY
AURÉLIE TACHENY, EPPENDORF APPLICATION TECHNOLOGIES S.A., NAMUR, BELGIUM

Introduction

Consistency in the performance of cell-based assays is crucial in order to obtain reliable data. The edge effect is a common problem in 96-well formats leading to variances between read-outs of the outer wells compared to the inner wells of a plate. Increased evaporation from the peripheral wells is assumed to be associated with this edge effect phenomenon. A very common method to circumvent the edge effect is to avoid seeding cells in the peripheral wells at the expense of sample throughput and efficiency (Fig. 1).

The Eppendorf 96-Well Cell Culture Plate has an outer moat which can be filled with liquid in just one pipetting step. It has been shown previously that evaporation can thus be reduced [1]. Here we show that the Eppendorf 96-Well Cell Culture Plate not only prevents evaporation but also minimizes well-to-well variability in cell proliferation across the plate.

Materials and methods

Two standard cell viability assays were used. Experimental results were verified by three independent replicates. The outer moat of the Eppendorf plates was filled with 5 mL sterile water or a pre-warmed gelled solution (0.5 % agarose). The plate from competitor C was prepared according to manufacturer's instructions.

Prior to cell seeding the plates were equilibrated for 1 h under standard conditions in a Galaxy® CO_2 incubator. Cells were thawed and pre-cultivated for at least two passages before use. For the first experiment HeLa cells (100 cells/well) were seeded in a final volume of 100 µL/well.

For the second experiment A549 cells (900 cells/well) were seeded in a final volume of 100 µL/well. To minimize pipetting variances caused by manual pipetting, semi-automatic cell seeding was performed using the ep*Motion*® 96. Plates were incubated at standard culture conditions for seven days. HeLa cell proliferation was analyzed after seven days by WST-1 assay (Roche®) according to manufacturer's instructions. A549 cell proliferation was analyzed after four days by AlamarBlue® assay (Invitrogen®) according to manufacturer's instructions.

Results and discussion

During longer incubation periods in 96-well formats, evaporation especially in the outer wells becomes apparent, because these wells are not completely surrounded by neighboring wells. Here we show that the insulation of edge wells minimizes the edge effect and positively affects the well-to-well consistency.

In the first experiment we compared the Eppendorf Cell Culture Plate with standard cell culture plates.

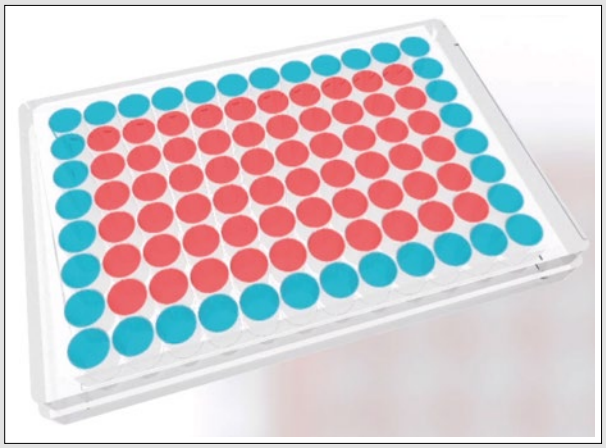


Fig. 1: A common way to prevent the edge effect is exclusion of the outer wells of a 96-well plate. This leads to a decrease of 38 % per plate regarding assay throughput.

In the second experiment we compared the Eppendorf plate with a plate that has four reservoirs that can be filled with liquid. In both experiments equal starting cell numbers were used, and cells were grown for several days. Viable cell count was analyzed by either WST-1 or Alamar blue assay.

As seen in Fig. 2 the edge effect affects all tested competitor plates.

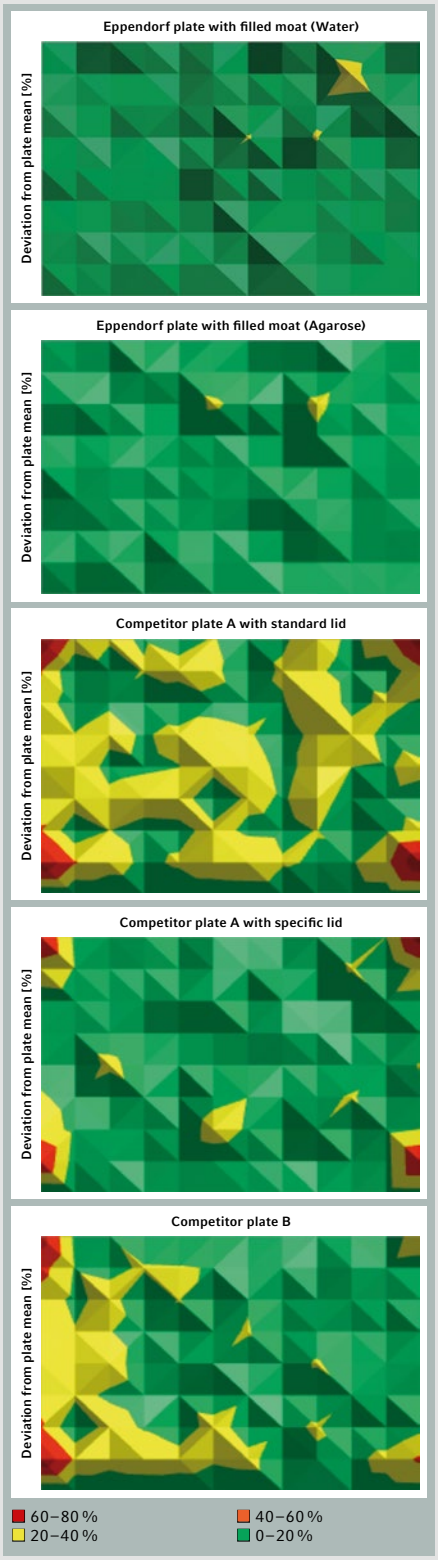


Fig. 2: Comparison of cell proliferation consistency across the plate expressed as percent deviation from plate mean

Superior Well-to-Well Consistency with Eppendorf Cell Culture Plates

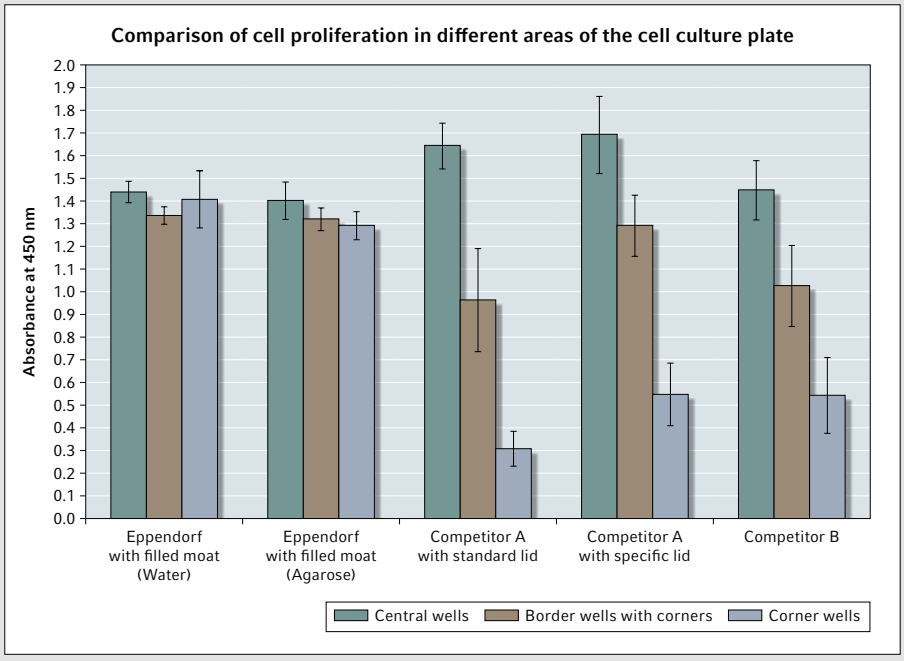


Fig. 3: Comparison of cell proliferation in different areas of the cell culture plate

Plates from supplier A and B show a strong edge effect. In the corner wells deviations of >70 % from the plate mean can be observed. Even in the center wells cell number variations of >30 % from plate mean are visible. The competitor plate A with a specific evaporation-reducing lid shows more homogeneous cell growth across the plate, but the edge effect is still severe with a deviation from the plate mean of >60 %. Only the Eppendorf plate with the filled outer moat shows a homogeneous well-to-well consistency in cell proliferation across the entire plate including the edges.

The moat of the Eppendorf Cell Culture Plate can be filled with either sterile liquid (e.g. water or buffer) or agarose solution (0.5 %). Both options effectively reduce the well-to-well variances across the plate. A gelled solution can offer a handling advantage and the possibility to prepare the plates ahead of the experiments.

Fig. 3 clearly shows that cell growth variability in all areas of the plate is minimized in the Eppendorf Cell Culture Plate whereas in the competitor plates, cell growth in the border and edge wells is impaired by the edge effect.

In the second experiment we compared the Eppendorf Cell Culture Plate with a plate that is equipped with four reservoirs that can be filled with liquid. Both plates prevent the edge effect and reduce growth deviations over the plate. But in the Eppendorf Cell Culture Plate only 5 % of the wells show deviations above 10 % whereas up to 9 % of wells are affected in the tested competitor plate.

The maximum deviation in the Eppendorf plate is only 12 %. The maximum deviation in the Competitor plate C at 21 % is nearly twice as high as in the Eppendorf plate (data shown in the original Application Note 384).

Conclusion

Filling the outer moat, either with a liquid or a gelled solution (like 0.5 % agarose), leads to a superior well-to-well consistency in cell growth in the Eppendorf Cell Culture Plate. By this, the phenomenon of the edge effect can be minimized leading to reproducible performance in cell-based assays.

Literature

[1] Wagener J, Plennevaux C: Eppendorf 96-Well Cell Culture Plate – A simple method of minimizing the edge effect in cell based assays. Eppendorf Application Note 326; www.eppendorf.com/appnote326

The complete version of this Application Note can be downloaded in PDF format at www.eppendorf.com/appnote384 *or using the QR-code.*

Eppendorf BioSpectrometer® kinetic: Activity Measurements of Enzymes Immobilized on a Silica Matrix

MICHAEL DREIFKE, MICHAEL FRÖBA, UNIVERSITY OF HAMBURG, INSTITUTE OF INORGANIC AND APPLIED CHEMISTRY, GERMANY
MARTIN ARMBRECHT, EPPENDORF AG, HAMBURG, GERMANY

Abstract

For this report, we studied the possibility of performing activity measurements of enzymes immobilized on a silica matrix using the Eppendorf BioSpectrometer kinetic. It is more common to measure free enzymes. However, immobilization of enzymes is expected to achieve increased stability and to offer the possibility of recovering the enzymes for further measurements. We could show that activity determination using immobilized enzymes is possible.

Since the Eppendorf BioSpectrometer allows direct calculation of specific enzyme activity (U/mg), additional time-consuming calculations are no longer required. The temperature-regulated cuvette shaft supports the precise maintenance of a constant temperature, which is required for enzyme activity measurements.

Introduction

Due to their high substrate specificity, enzymes are gaining importance in the field of chemical synthesis. However, an enzyme which is solubilized in the reaction medium is difficult to separate from the reaction properly, thus considerably limiting the possible use of enzymes, as well as inflicting additional cost. Immobilization of the enzyme on a porous carrier material can solve this dilemma. Hereby, the pore forms a protective cavity for enzyme from external influences and can thereby stabilize it [1]. Not only are immobilized enzymes easily removed from a reaction mixture, but the immobilizate can be re-used.

In the present work, a 6-phospho gluconate dehydrogenase (6PGDH) is immobilized on a mesoporous silica matrix. This enzyme catalyzes oxidation of 6-phospho gluconate to ribulose-5-phosphate in the pentose phosphate pathway (Fig. 1).

In this step of the reaction, one equivalent of $NADP^+$ is reduced to NADPH ($\lambda_{max} = 340$ nm) per one equivalent of 6-phospho gluconate.

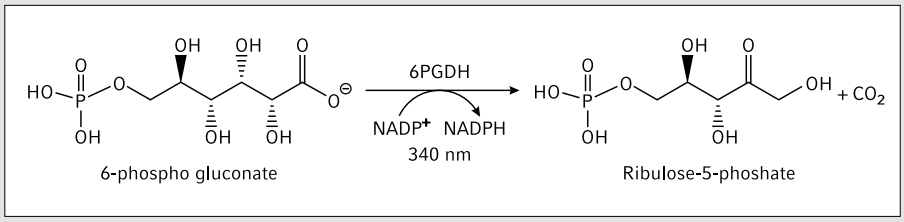


Fig. 1: 6PGDH-catalyzed oxidation of 6-phospho gluconate to ribulose-5-phoshate and CO_2 with concurrent reduction of $NADP^+$ to NADPH

Therefore, enzyme activity can be detected photometrically by an increase in absorbance at a wavelength of 340 nm over a pre-determined period. For a detailed description of the experiment and the methods used for quantification and activity measurements in the BioSpectrometer kinetic, see Eppendorf Application Note 370 [2].

Methods

Activity measurement

For the activity measurement in the BioSpectrometer kinetic it is possible, via programming a factor, to calculate the enzyme activity in the desired dimension directly after the measurement (Fig. 3). An additional reduction of working time during enzyme activity measurements is the possibility of subsequent adaption of the range which is applied for calculating the enzyme activity. In this case, especially the linear range of the measuring curve, which is used for the following activity measurement, is important.

In Fig. 4 the measurement without (Fig. 4a) and with (Fig. 4b) adaption to the linear range is displayed.

Protein quantification using the Bradford method

Calibration of the BioSpectrometer kinetic for protein quantification according to Bradford was performed using a standard series of bovine serum albumin (BSA) in a concentration range between 0.025 mg/mL and 2 mg/mL, measured at $\lambda = 595$ nm (Table 1).

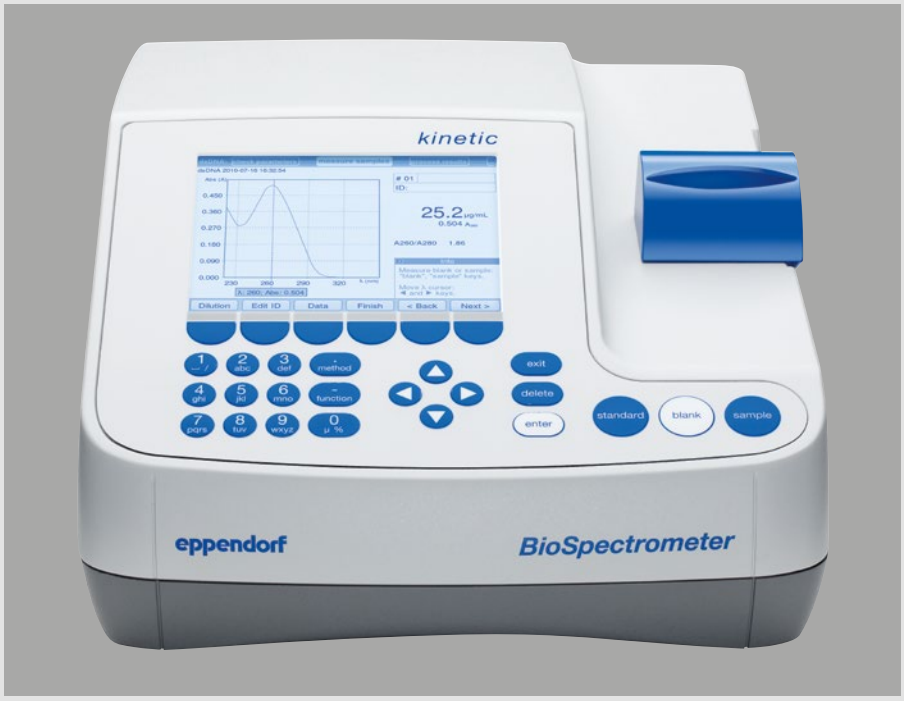


Fig. 2: Eppendorf BioSpectrometer kinetic

Eppendorf BioSpectrometer® kinetic: Activity Measurements of Enzymes Immobilized on a Silica Matrix

Fig. 3: Input of the factor into the method parameters for direct determination of specific enzyme activity (U/mg)

Fig. 4a: Preliminary measurements of enzyme activity: linear regression without adjustment

Fig. 4b: Preliminary measurements of enzyme activity: linear regression with adjustment

Fig. 5: Analysis of the standard curve for determination of protein concentration using the Bradford method using cubical regression. Red box: regression accuracy per coefficient of determination R^2

Fig. 6: Determination of specific enzyme activity of 6-phospho gluconate dehydrogenase

Standard	*BSA concentration (mg/mL)*
1	0.025
2	0.125
3	0.25
4	0.5
5	0.75
6	1
7	1.5
8	2

Table 1: BSA dilution series for the standard curve used in the Bradford assay

The Eppendorf BioSpectrometer allows adjustment of the regression analysis during generation of the standard curve, whereby several different regression analyses are offered via the function "curve fit". Accuracy of the regression is expressed by the coefficient of determination R^2 (Fig. 5).

Results and discussion

Using the pre-measurement, the measuring range for the activity detection could be restricted significantly, which meant an extensive time saving. In Fig. 6 an example of the result is shown.

It could be demonstrated that the Eppendorf BioSpectrometer kinetic is ideally suited to perform photometric activity assays with immobilized enzymes. The temperature-regulated cuvette shaft of the BioSpectrometer kinetic reduced the risk of temperature variations, thus achieving a higher reproducibility in sensitive enzyme assays.

The function "linear regression" allows exact definition of the linear range of any activity assay. Input of a conversion factor in the method parameters enables direct calculation of enzyme activity, thus immediately saving valuable time as additional calculation steps are not required. This becomes crucial when multiple successive measurements are involved.

Literature

[1] Fried DI, Bednarski D, Dreifke M, Brieler FJ, Thommes M, Fröba M: Influence of the hydrophilic-hydrophobic contrast of porous surfaces on the enzymatic performance. *J. Mater. Chem. B*, 2015,3, 2341-2349.

[2] Dreifke M, Fröba M, Armbrecht M: Eppendorf BioSpectrometer® kinetic: activity measurements of enzymes immobilized on a mesoporous silica matrix – Eppendorf Application Note No. 370 (2016).

Download at www.eppendorf.com/appnote370 or use the QR-Code.

NATASCHA WEISS, EPPENDORF AG

Better Safe Than Sorry: Verification of Spectrophotometers

Will high-quality spectrophotometers still produce accurate and precise measuring results after longer periods of use? Even laboratories which do not work in accordance within specific regulations have good reasons to have their devices tested. Environmental influences, wear and tear, damage, and dirt may impair instruments and lead to incorrect results. Regular checks can identify such possible impairments and thus avoid unnecessary repetition of work and associated costs [1].

Proper performance verification of spectrophotometers is carried out using certified and traceable reference materials that can be traced back through regular comparative measurements to a standard at a metrological institute via a continuous, uninterrupted chain.

During verification, the measurement values of an instrument are compared to the nominal value of the reference standard, and deviations are noted and documented. The deviations must remain within defined limits which are based on the nominal value of the reference standard, but which also take into consideration the technical data of the instrument.

It is possible for users to carry out verifications of their photometers themselves using the respective certified reference materials. Alternatively, verification can be performed by a qualified service company.

Solutions by Eppendorf

Photometric self test

The Eppendorf BioPhotometer® D30 and the Eppendorf BioSpectrometer® models feature an integrated self test. When activated, the instrument automatically tests the functionality of the photometric unit.

Reference filter sets

Eppendorf offers certified UV/Vis reference filter sets for verification of photometric and wavelength accuracy (Fig. 1).

The software of Eppendorf instruments has a program for performing this test [2]. In addition to being displayed on the screen (Fig. 2), the generated data can be printed for documentation purposes, or exported as a PDF file.

If reference materials which have been certified by an accredited body need to be used, the instruments can be tested, e.g., by using the DAkkS* certified filters by Hellma® Analytics in combination with Eppendorf Control Charts [3,4] (*Deutsche Akkreditierungsstelle GmbH, Berlin, Germany).

Eppendorf service

Eppendorf offers different epServices performance plans for photometry which include preventive maintenance as well as certification services (www.eppendorf.com/epServices).

Fig. 1: Eppendorf Reference Filter Set for checking photometric and wavelength accuracy of the Eppendorf BioSpectrometers basic and kinetic

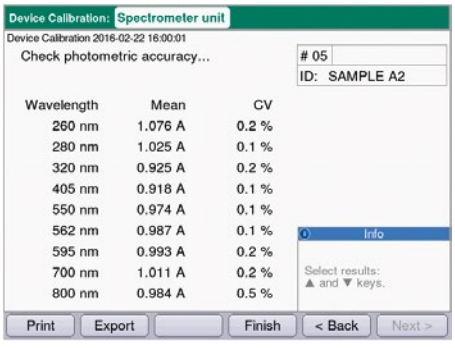


Fig. 2: Eppendorf BioSpectrometer – data display during calibration with the Eppendorf Reference Filter Set

References

[1] Weiss N. Better safe than sorry: Verification of spectrophotometers for accurate and reliable measuring results. Eppendorf White Paper 33; www.eppendorf.com/whitepaper33

[2] Armbrecht-Ihle M, Borrmann L. Evaluating the functionality of Eppendorf BioPhotometer® and Eppendorf BioSpectrometer® using a Secondary UV-VIS Filter Set. Eppendorf Userguide 10; www.eppendorf.com

[3] Weiss N. Verification of the Eppendorf BioSpectrometer® with Hellma® Filter F1 using a control chart. Eppendorf Short Protocol 019; www.eppendorf.com

[4] Weiss N. Verification of the Eppendorf BioSpectrometer® with Hellma® Filters F2, F3, F4 using a control chart. Eppendorf Short Protocol 020; www.eppendorf.com

Documents 2–4 can bei found at the Eppendorf homepage under Service & Support within our comprehensive Knowledge Base.

TANJA MUSIOL, EPPENDORF AG

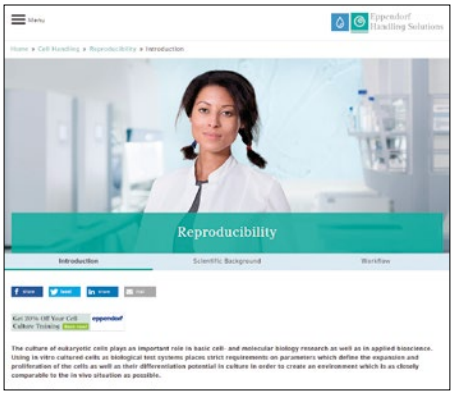
Proper Handling of Cells in the Lab (III)

After dedicating previous issues of BioNews to the topics "Prevention of contamination in the cell culture laboratory" and "Cell identification", in this edition, we will examine the basic conditions that must be met to safeguard reproducible results in cell culture. This topic is especially crucial, as cell culture work is not governed by strictly defined quality standards.

Due to a lack of standards and established guidelines for "Good Cell Culture Practice", differences exist between the modes of operation from one laboratory to the next, and sometimes even between different people working in the same laboratory. Such circumstances make it difficult to compare different experiments and experimental results. Only if parameters are truly equivalent, the comparison of experimental set-ups will make sense.

For this reason, increasing numbers of current publications are dedicated to a definition of guidelines, covering topics such as:

> Characterization and documentation of critical features of cell lines used
> Reliable quality assurance for traceability and reproducibility
> Recording and scientific documentation of experimental data
> Education and training of staff with respect to basic cell culture practice, specific processes, questions of safety as well as ethical aspects



From these recommendations, Standard Operating Procedures (SOPs) may be derived, which describe, in writing and step by step, the process and the workflow in the laboratory, for the purpose of ensuring accuracy and precision during the work processes, which, in turn, are necessary for preserving quality and reproducibility. Among other recommendations, it is deemed important to summarize the basic parameters for each cell line that is used in the cell culture laboratory, including information regarding its identity and culture conditions, as well as the daily work steps involved. Continuous culture of a cell line requires continuous record-keeping and documentation.

Additional information

On our new website with the topic "Cell Handling" you will find, along with other information, a template which may be used for clear and consistent recording of the most important details relevant to the cultivation of each cell line. Furthermore, you will find extensive links and references all about this topic area.

More details at
www.eppendorf.com/cellexperts

Tip

A Video Journey Through the Laboratory

Are you familiar with the varied selection of Eppendorf products for applications in the modern molecular biology laboratory? Yes? No? Maybe? With our new video, we will take you along on a journey through the laboratory, stopping at different stages of the workflow to show you close-ups as well as detailed views of individual products.

During this journey, we are focusing especially on those product features that can help you simplify daily routine processes in the laboratory. The specially designed Eppendorf Kit rotor® for centrifuges enables nucleic acid purification in spin columns without running the risk of losing the tube lids during centrifugation. The easy to clean microliter measuring cell Eppendorf µCuvette® G1.0 allows nucleic acid quantification of even the smallest volumes – in a standard spectrometer. Experience how comfortably all the wells of a 96-well plate may be filled or how smart features can facilitate daily use of a freezer.

Meet the products by Eppendorf today and experience them from an entirely new perspective while gaining an overview of the equipment within the different work areas.

The video is available at
www.eppendorf.com/workflows.

CARSTEN BUHLMANN, EPPENDORF AG

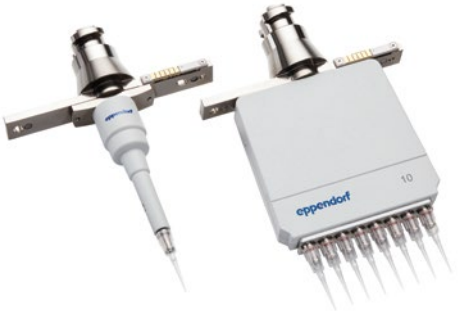
ep*Motion*® 10 µL System: Improved Pipetting of Sub-Microliter Volumes

Reducing sample and reagent volumes can lower costs and enable more experiments and projects. However, downscaling of applications like NGS library preparation or PCR set-up will require high precision and accuracy of sub-microliter pipetting to achieve the same experimental reproducibility as with larger volumes. Equipped with the new 10 µL system, the ep*Motion* is one of the most precise and accurate automated liquid handling systems available and a valuable tool for labs wanting to increase throughput and consistency of results.

Automated NGS library preparation

There is a growing list of methods for NGS library preparation kits from various suppliers that can be run on the ep*Motion* (see table). Automating labor-intensive steps like pipetting, mixing, temperature control, and magnetic separation reduces hands-on time and eliminates errors associated to manual handling. The extension towards smaller pipetting volumes thus opens up new opportunities for downscaling NGS library preparation and yielding significant cost savings.

The recently introduced 10 µL system for ep*Motion* 5070, 5053, and 5075 facilitates pipetting of small volumes from 0.2–10 µL. It comprises two new dispensing tools: the TS 10 single-channel and the TM 10-8 eight-channel, dedicated epT.I.P.S.® Motion (in seven variants), and a new 10 mL reservoir. The new epBlue™ 40.5 software supports the update of current ep*Motion* units to the 10 µL system.



Small volume ep*Motion* dispensing tools TS 10 and TM 10-8 for 0.2–10 µL

Available ep*Motion*® methods for NGS library prep kits (Status: April 2017)

Supplier	Kit Name	Status
Illumina®	TruSeq® Stranded Total RNA Sample Preparation Kit	Illumina® Qualified*
Illumina®	TruSeq® Stranded Total mRNA Sample Preparation Kit	Illumina® Qualified
Illumina®	TruSeq® Nano DNA Sample Preparation Kit	Illumina® Qualified
Illumina®	TruSeq® DNA PCR-free Sample Preparation Kit	Illumina® Qualified
Illumina®	TruSeq® Rapid Exome Library Prep kit	Illumina® Qualified
Illumina®	TruSeq® RNA Access Library Prep kit	Illumina® Qualified
Illumina®	Nextera® Rapid Capture Exome	Illumina® Qualified
Illumina®	Nextera® Rapid Capture Custom Enrichment/TruSight® Cancer	Illumina® Qualified
Illumina®	TruSight® Tumor 15	Illumina® Qualified
Illumina®	Nextera® XT Library prep kit	In qualification progress
Illumina®	TruSight® HLA Sequencing Panel	In qualification progress
Illumina®	TruSight® RNA Pan-cancer Panel	In qualification progress
Illumina®	TruSeq® Custom Amplicon Low Input	In qualification progress
Illumina®	TruSight® RNA fusion panel	In qualification progress
Illumina®	ChIP-Seq DNA Sample Prep Kit	Customer Qualified
Illumina®	TruSight® One Sequencing Panel	Customer Qualified
Illumina®	ForenSeq® DNA Signature Prep	Customer Qualified
KAPA® BioSystems	KAPA® Hyper Prep kit	KAPA® Qualified
KAPA® BioSystems	KAPA® HyperPlus kit	KAPA® Qualified
KAPA® BioSystems	KAPA® HTP Library Preparation kit	Customer Qualified
KAPA® BioSystems	KAPA® LTP Library Preparation kit	Customer Qualified
New England Biolabs	NEBNext® Ultra™ DNA Library Prep Kit	Customer Qualified
New England Biolabs	NEBNext® Ultra™ II DNA Library Prep Kit	Customer Qualified
Agilent® Technologies	HaloPlex® Target Enrichment	Customer Qualified
Agilent® Technologies	SureSelect® XT Target Enrichment	Customer Qualified
Qiagen®	GeneRead® Panel	Customer Qualified
Qiagen®	QIAseq® FX DNA Library Kit	Customer Qualified
Thermo Fisher Scientific®	Ion AmpliSeq® NGS Panels	Customer Qualified

*Qualified means the libraries perform comparably to those prepared manually.

More information at **www.eppendorf.com/automation**

BERRIT HOFF, EPPENDORF AG

Stay Informed – On All Channels

How do you obtain your information? Do you still read from paper (attention: old school … see column), or do you use PC, smartphone, tablet, or phablet? Communication experts worldwide are constantly studying the most recent trends pertaining to the acquisition, conveying, and processing of information. Latest discoveries are hyped in close succession, only to be rapidly overtaken by even more recent announcements.

These phenomena are joined by continual technological advances – not to mention virtual, augmented, and other realities.

As a company that prides itself in keeping our customers informed at all times – on as many channels as possible, with current and relevant content – we consider this fast-paced change a catalyst for continuous development of our information universe. In this article, we would like to present you with a number of innovations and highlights.

Eppendorf website – naturally responsive

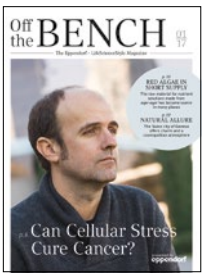
Whether you want to gather information or order a product from your local eShop – thanks to responsive design, the highest possible user-friendliness is guaranteed on all mobile end devices: www.eppendorf.com.

Eppendorf Handling Solutions

Within this new online area with its focus on Liquid Handling, Cell Handling, and Sample Handling, you will find applications tips and facts, current reviews of new scientific findings, videos, and online games, as well as dates of upcoming training sessions and webinars: handling-solutions.eppendorf.com.

Stay Informed infographics

The infographics of our new Stay Informed series depict clearly, in word and image, the prerequisites of common laboratory technologies that are crucial in ensuring reproducible results. Available as a reference and for downloading at www.eppendorf.com/stayinformed.



A bit of life style for your lab

Relaxation away from the stressful daily laboratory routine is at your fingertips: in the LifeScienceStyle magazine "Off the Bench", published twice a year, we collect exciting and entertaining information from the laboratory scene, conduct interviews, and explore new cities. Read online or subscribe at www.eppendorf.com/otb.

Eppendorf on YouTube®

The Eppendorf channel on YouTube® has existed for over 10 years, showing many step-by-step instructions and tutorials: www.youtube.com/eppendorf.

Social media

Of course, Eppendorf is also represented on Facebook®, LinkedIn®, and twitter®. This is where we post current news and establish contact with a growing number of followers:

> facebook.com/eppendorf

> linkedin.com/company/eppendorf-ag

> twitter.com/eppendorf_ag

News

Clash of Readers

Lunch break in an Eppendorf tea lounge. Ms. H. is reading a book. It has been quiet so far, but now the colleagues are returning from the cafeteria.

Colleague X.: "You still read books? That's odd."

Ms. H. (looks up): "Why not? It feels good to hold, the cover is beautifully designed, and somehow a book has something familiar about it. There really is no need for you to pity me."

Colleague Z interjects: "Folks, print is so completely old school. And how many trees do you think have to come down for books and newspapers? I only read online these days. Books are no longer welcome in my house."

A week later, Ms. H. is back in the tea lounge, this time with her e-book reader.

Colleague X.: "Hey, how long have you had that e-book reader? That's odd. I thought you were only reading books?"

Ms. H. (sighs): "Well, the world is not just black and white, is it? And, guess what, I don't only have this reader, but also a tablet for reading journals, and a smartphone for reading the news."

Colleague X.: "Wow, I guess I completely underestimated you!"

Ms. H.: "And what are you reading at the moment? Can I trust my eyes: a printed internet newspaper? That's odd … and a little old school, right?" *(smile!)*

Colleague X.: "Ahem, well, sometimes I enjoy holding paper in my hands. You don't have to upload anything, you can turn the pages, and there is no constant distraction. Like I said, quite pleasant from time to time. But I really should not be saying this, as an Onliner …"

Ms. H.: "Not to worry, I can keep a secret." *(More quietly):* "Most likely …"

SUSANNE VON DER KAMMER, EPPENDORF AG

Do Freezing and Cold Storage Impact Sample Quality?

To scientists, the quality of a biological sample is of paramount importance, as it is in fact the source material for all downstream experiments. In order to preserve the biological material in its optimal condition over a long period, the freezing process as well as subsequent storage at the lowest possible temperature are essential. A White Paper* by the author John M. Baust presents the scientific background on the processes that occur during cryopreservation.

Storage of biological sample material occurs at temperatures of –80 °C in ultra-low temperature freezers or –196 °C in liquid nitrogen, and it is dependent on the type of material and the storage time. The freezing process and long-term storage may impact negatively on the quality of the sample through physical influences as well as through molecular alterations of the material. Under these conditions, bacteria, viruses, and subcellular components, such as DNA, RNA, and proteins, are less vulnerable than mammalian cells or tissues, which may reveal a reduction in quality or viability only following thawing and further cultivation. In order to protect the cells during the freezing process, cryoprotective agents are added, which act to minimize cell membrane damage. Controlled cooling at a rate of –1 °C/min is imperative in order to avoid drastic volume changes of the cells during the dehydration process. Cellular dehydration continues up to the point at which the cell transitions to a glassy state; thus, sample degradation may continue to occur at storage temperatures above this transition point.

White Paper on cryopreservation

In his White Paper, John M. Baust presents the scientific background on the processes that occur during cryopreservation. He describes successful storage of bacteria, viruses, DNA, RNA, and proteins over 1–2 years and beyond.

It is important to maintain a constant final temperature; each thaw and freeze event or warming of the sample during storage may lead to a loss in quality.

Stable storage is therefore vitally dependent on an instrument that offers good insulation of the individual compartments with as little warming of the samples as possible and which keeps the loss of refrigeration to a minimum when a door is opened. For this purpose, Eppendorf offers ultra-low temperature freezers with a choice of three or five individual compartments, featuring separate inner doors and additional inner door insulation. A very effective cooling system ensures quick temperature recovery times following door opening, thus keeping energy consumption low.

*John M. Baust, Ph.D. Biopreservation: The Impact of Freezing and Cold Storage on Sample Quality; White Paper No. 031.

Download at www.eppendorf.com/whitepaper31 or scan the QR code.

Eppendorf ultra-low temperature freezers are known for their energy efficiency and robustness, as well as for their reliable long-term sample preservation. All details are available at www.eppendorf.com/freezers.

The flexible, soft polymer seal ensures exceptional interior space insulation.

CAROLYN TAUBERT AND BERRIT HOFF, EPPENDORF AG

Eppendorf Prize Winners 2016/2017: Gilad Evrony & Tom Baden

eppendorf & Science PRIZE FOR NEURO BIOLOGY

The Israeli-American scientist Gilad Evrony, M.D., Ph.D. has won the 2016 *Eppendorf & Science Prize for Neurobiology* of 25,000 USD for his work on developing technologies to sequence and analyze the genomes of single cells from the human brain. Dr. Evrony's research, performed at Boston Children's Hospital and Harvard Medical School with Dr. Christopher Walsh and colleagues, has revealed a diversity of mutations in neuronal genomes indicating that every neuron in the brain carries a unique fingerprint of somatic mutations. Such mutations can cause focal brain malformations and may have a role in other unsolved neurologic diseases. The technology also allows, for the first time, reconstruction of developmental lineage trees in the human brain to study how cells proliferate and migrate to build the brain.

Dr. Evrony is currently pursuing clinical training in pediatrics at Mount Sinai Hospital in New York and continuing his research developing novel technologies for studying the brain and neuropsychiatric diseases.

More information at www.eppendorf.com/prize

EPPENDORF AWARD FOR YOUNG EUROPEAN INVESTIGATORS

The 2017 *Eppendorf Award for Young European Investigators*, endowed with € 20,000, went to Tom Baden PD., Ph.D. (Senior Lecturer in Neuroscience at the School of Life Sciences, University of Sussex, Brighton, United Kingdom) for his ground-breaking work on signal processing in the retina. "Tom Baden's results have profoundly changed our understanding of circuits and synaptic computation in the retina, revealing novel and exciting properties of sensory neurons", the jury chaired by Prof. Reinhard Jahn (Max Planck Institute for Biophysical Chemistry, Göttingen, Germany) concluded. "These findings are of general significance for our understanding how small neuronal microcircuits can dissociate complex sensory patterns into specific representations within the nervous system."

More information at www.eppendorf.com/award

Both prize winners will visit Eppendorf in Hamburg during 2017. Check out the next BioNews issue for more info!

Win a Pipette 3-Pack!

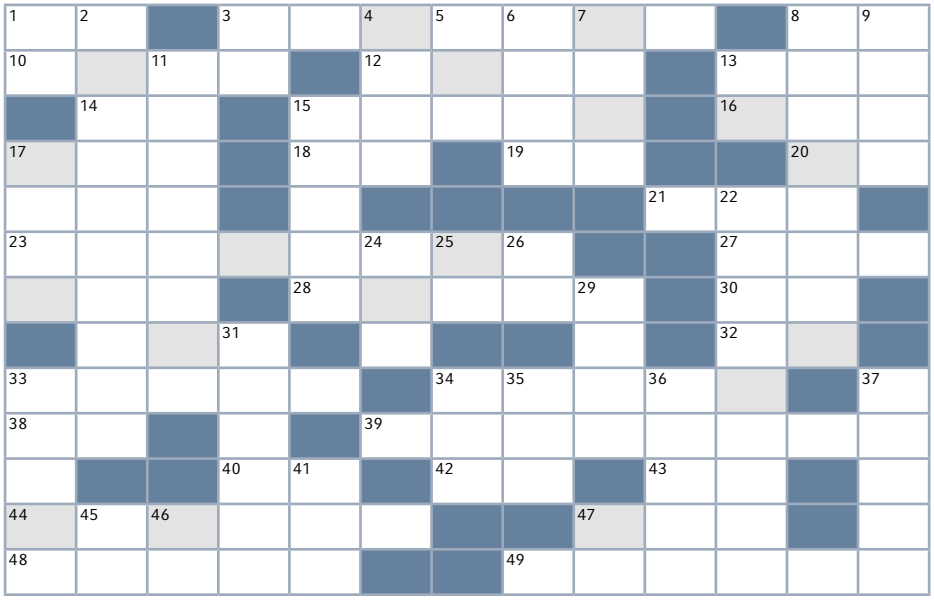
The solution of the prize competition of BioNews No. 45 was "Pipetting with a Smile". Ilka Werner-Martini (Ruhr University Bochum, Germany) won the first prize.

Good luck in our new competition!

How to find out the solution: Simply arrange all letters in the light gray boxes of the crossword in the correct order. Send us the solution until **October 31, 2017**.

You can either send us an e-mail to **bionews@eppendorf.de**, or participate online at **www.eppendorf.com/bn-service**.

All correct answers will be considered for a prize. 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. 49.



1st Prize:

3 Eppendorf Reference® 2 (in a 3-Pack of your choice)

2nd to 5th Prize:

1 Amazon® Voucher worth 50.00 EUR

6th to 15th Prize:

400 bonus epPoints® each

(epPoints registration required)

ACROSS

1 Plumbum (abbrev.)
3 Gift or statement given as an expression of gratitude or esteem
8 Chemical symbol for barium
10 Actors in a play or movie
12 Latin prefix meaning "half"
13 Russian space station
14 US postal abbreviation for Connecticut
15 Universe of the Hunger Games (novel series)
16 School of buddhism
17 Jamaican music style
18 Organelle with network of membranes in eukaryotic cells (abbrev.)
19 ISO country code for the United Arab Emirates
20 007 opponent, holds a doctor's degree
21 Transaction number in electronic banking
23 Major form of pop music in the 60s and 70s
27 Island in the Irish Sea
28 City in the south of France
30 ISO country code for Sierra Leone
32 Chemical symbol for tellurium
33 Not forbidden
34 Head of Slytherin (surname)
38 Internet slang for "You Are"
39 Famous school of witchcraft and wizardry
40 ISO country code for Morocco
42 I, myself in Spanish
43 Parkinson's disease (abbrev.)
44 Movie theater
47 Aromatic beverage
48 Pupil at 39 across (first name)
49 Person who cultivates land

Down

1 Programmable electronic device (abbrev.)
2 Low-cost traveller
3 ISO country code for Trinidad and Tobago
4 River flowing through Munich (Germany)
5 Male given name
6 Town in northern Sweden
7 Something one can never get back
8 Cultural event that happens every two years
9 River in Tuscany
11 Term indicating the most important performers in a movie
13 Maputo is the capital of this country (ISO country code)
15 Ancient Jordanian city
17 Neighborhood in Manhattan
22 Capital of the Netherlands
24 Mork's home planet
25 10 mL are equal to one of these (abbrev.)
26 ISO country code for Kenya
29 Salad with shredded cabbage
31 Hand tool
33 Meal eaten at midday
34 High protein vegetable
35 Non-governmental organization (abbrev.)
36 Beats rock
37 Academy Award
41 Female given name
45 US postal abbreviation for Iowa
46 ISO country code for Nauru
47 Chemical symbol for tantalum

Solution hint for prize competition of BioNews No. 47

		A						N				E			I	

Send us the solution until **October 31, 2017**, via e-mail to **bionews@eppendorf.de**, or participate online at **www.eppendorf.com/bn-service**.