BIO NEWS
No. 49 – 2018

eppendorf

Centrifuges: Everything Revolves around Your Workflows

> VisioNize® system: labwork meets network
> ViscoTip®: new specialist for tough-to-handle liquids
> Proper handling of cells in the laboratory (IV)

Application Notes

Ready-to-use CCCadvanced™ FN1 motifs surface for xeno-free expansion of hiPS cells · ViscoTip®: optimized performance for highly viscous liquids · etc.


CHO Cell Culture in
Eppendorf BioBLU® 3lc Single-Use Vessels

Dear Readers,

Here we are again with new insights and products updates from Eppendorf.

This year at Eppendorf, (nearly) everything revolves around centrifugation. Two brand new centrifuges, Centrifuge 5910 R and Centrifuge 5425, are ready to conquer their places in the labs of the world! Read more on pages 4–5.

Are you working with challenging liquids, e.g. highly viscous or thick, sticky solutions? We knew there was demand for better solutions for pipetting such liquids, but we were overwhelmed by the positive feedback to our new ViscoTip® at various tradeshows. This new dispenser tip for use with the Multipette® (U.S./CAN: Repeater®) will be your new problem solver! Learn more on page 6 and Application Notes 5–6).

Eppendorf sees digitalization and networking as two of the most important future market requirements. The VisioNize® system allows users to centrally monitor a variety of Eppendorf instruments at the same time (page 11). Many of our new products can be networked with VisioNize: the Mastercycler® X50 thermal cycler (page 11), the Innova® S44i biological shaker (page 9), and the CellXpert® CO_2 incubator (page 10), to name just a few.

We are still celebrating 25 Years of BioNews! In our anniversary prize competition sequel you can win one of 5 "personalized" pipettes with laser engraving. Good luck!

Many other reports and four new Application Notes on diverse topics round off this issue. We hope you like it!

Your Eppendorf BioNews Team

PS: Would you like to share your thoughts with us regarding 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

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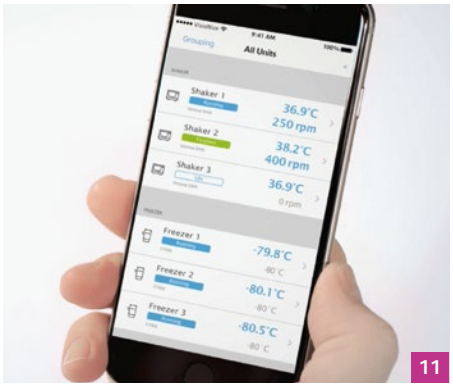


Eppendorf BioNews Application Notes

CHO Cell Culture in
Eppendorf BioBLU® 10c Single-Use Vessels

AMANDA SUTTLE AND MA SHA, EPPENDORF INC., ENFIELD, CT, USA

FLORIAN BUNDIS, EPPENDORF AG

Centrifugation: Everything Revolves around Your Workflows

Centrifugation is an integral component of many workflows in modern scientific and diagnostic laboratories. The spectrum of methods ranges from pelleting precipitated DNA in the field of molecular biology to purification of lymphocytes and monocytes via density gradient centrifugation in the areas of cell biology and human diagnostics. Eppendorf started off the year by introducing two brand new centrifuges. Both instruments follow the same extremely high Eppendorf standards in reliability, safety, and convenience – the performance you need for your successful lab applications.

With more than 50 years of experience …

In 1964, Eppendorf introduced the first microcentrifuge for laboratory applications to the market. The Model 3200, with its fixed rotational speed and only one dial to select run time, was still relatively simple in terms of construction. Together with the microliter pipettes and "Eppi®" tubes that had been newly introduced only a short time before, the Model 3200 was an integral component of the Eppendorf microliter system. This system enabled work with even the smallest of sample volumes, thus revolutionizing biomedical research worldwide.

… and today's power of innovation …

Today, Eppendorf develops innovative, high-quality centrifuges for a broad range of applications. They offer an ergonomic operating concept with a multitude of options and they adhere to the highest safety standards. Refrigerated centrifuges are further equipped with an advanced temperature management for the protection of your sensitive samples from heat. It is the goal of Eppendorf product development to surpass the expectations of our customers, to simplify laboratory processes, and to provide our users with future-oriented new technologies.

… for the laboratories of tomorrow

This year, Eppendorf is introducing not one, but two new centrifuges. The microcentrifuges with up to 24 places are entering a new generation, where the completely newly developed non-refrigerated Centrifuge 5425 is replacing the legendary "laboratory standard", the Centrifuge 5424. Within the segment of large refrigerated high-capacity benchtop centrifuges, Eppendorf is expanding its product range. The Centrifuge 5910 R is a particularly versatile variant with a completely newly developed adapter concept for even quicker loading.

The new non-refrigerated Centrifuge 5425 is replacing the legendary "laboratory standard" Centrifuge 5424.

Centrifuge 5425 – My lab. My Centrifuge.

The Centrifuge 5425 has everything that you so loved and appreciated about its predecessor while at the same time offering additional new amazing features to make lab work even more enjoyable. For example, during the development of this 24-place microcentrifuge, special attention was paid to ensuring extremely low noise emission. Short runs of up to 12 minutes are whisper-quiet at an average of 45 dB(A). This length of time is sufficient for the centrifugation steps required by most DNA and RNA isolation kits. Only during longer runs will the ventilator switch to full capacity to dissipate heat from the instrument – but even then, the Centrifuge 5425 remains the quietest non-refrigerated microcentrifuge on the market.

Swing-bucket rotor for 96 PCR tubes

With respect to versatility, too, the Centrifuge 5425 is setting new standards. For the very first time a swing-bucket rotor accommodating 96 PCR tubes, for

The refrigerated Centrifuge 5910 R was designed to accommodate a particularly broad spectrum of applications.

quick centrifugation of droplets and condensate before and after PCR/qPCR, is available for a microcentrifuge. When harvesting bacteria and yeast, a new fixed-angle rotor for 5 mL tubes with screw or snap caps is ready for you.

Centrifuge 5910 R – the next benchmark

With its versatility and capacity, the refrigerated Centrifuge 5910 R is setting the next benchmark for benchtop centrifuges. With a comprehensive portfolio of fixed-angle and swing-bucket rotors, this centrifuge was designed to accommodate a particularly broad spectrum of applications.

Cell harvests in bottles of capacities up to 750 mL, large-scale DNA and RNA isolations as well as Ficoll® gradients for the purification of lymphocytes and monocytes are merely a few examples of the many areas of application.

The new universal swing-bucket rotor with unique universal adapters facilitates even quicker loading. This system allows centrifugation of conical vessels up to 50 mL, plates, and 250 mL bottles for the first time without the need to change rotors, rotor buckets, or adapters.

For the protection of your samples, the performance of the cooling system will always adapt dynamically to individual requirements, maintaining the stability of the selected temperature at all times. In addition to the FastTemp function for super-fast pre-cooling, the Centrifuge 5910 R offers FastTemp pro®. Pre-cooling starts automatically at a pre-programmable time – the centrifuge will already be pre-cooled when you arrive at the lab in the morning.

The new universal swing-bucket rotor with especially versatile adapters

Fit for the future

Today, after more than 50 years, the name Eppendorf more than ever stands for innovative, reliable, and future-oriented centrifuges that have proven themselves during long-standing service and that will continue to support you and your laboratory work – today and in the future!

More information on the Centrifuge 5425 is available at **www.eppendorf.com/my-lab-my-centrifuge**

Scan the QR code for more information!

More information on the Centrifuge 5910 R is available at **www.eppendorf.com/next-benchmark**

Scan the QR code for more information!

Close-up

FastTemp … what? FastTemp pro®!

In addition to the well-known FastTemp function for super-fast pre-cooling, Centrifuge 5910 R includes the new FastTemp pro software option. This feature allows for automated pre-cooling based on pre-programmable time and date. Simply turn the centrifuge into standby mode when you leave your lab and let FastTemp pro take care of pre-cooling in the morning so you don't have to. This saves you more than 90 % of energy overnight and programming is super easy.

Conclusion

Centrifuge 5910 R features a powerful state-of-the-art refrigeration system with advanced temperature management to keep your samples safe. Its automated pre-cooling function FastTemp pro allows for a better and more efficient use of time.

Watch our short video tutorial and learn how easy FastTemp pro programming is! Visit **www.eppendorf.com/fast_temp_pro**

Scan the QR code for more information!

By the way: Centrifuge 5920 R and Centrifuge 5430 R, too, feature the convenient FastTemp pro function! More information at **www.eppendorf.com/centrifugation**

CHRISTIAN HABERLANDT, EPPENDORF AG

Let it Flow! The New ViscoTip® for Highly Viscous Liquids

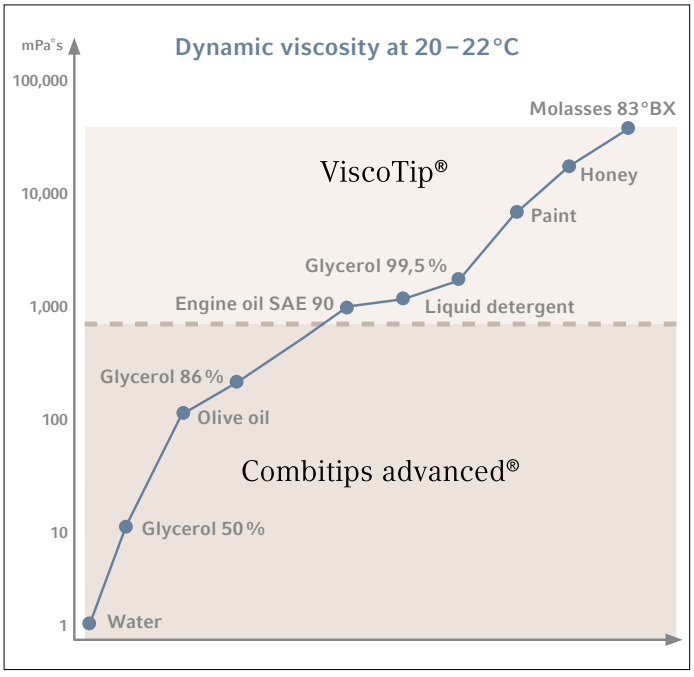
Processing highly viscous liquids such as glycerol 99.5 %, collagen, nail polish, creams, oils, or liquid honey in the milliliter volume range is a difficult task for both research and analytical laboratories. Usually, researchers depend on suboptimal tools such as spoons. This slows down the transfer of thick, sticky liquids from one vessel to another and impedes precise volume measurements and mixing processes. Learn here how the new ViscoTip facilitates processing of tough-to-handle, highly viscous liquids.

In order to process aqueous liquids precisely, modern laboratories use pipettes with different functional principles, each equipped with the respective consumable tips. For viscous liquids in particular, so-called direct displacement systems, which function similar to a syringe, have prevailed. One example is the Eppendorf Multipette® (U.S./CAN: Repeater®) family of handheld dispensers with the matching Combitips advanced® dispenser tips.

Increasing liquid viscosity, however, leads to growing flow resistance and adhesion forces inside the tip. This resistance will further increase with the volume to be processed. The consequence: Either the hand strength of the operator, the material of the operating levers, or the motors of electronic pipettes will reach their limit. Additionally, the processing speed will decrease with increasing viscosity, particularly if precision is of the essence. When Combitips advanced with 10 mL volume are used, the limit of the dynamic viscosity, which is dependent on the capacity of the pipetting system or the operator, respectively, consistently ranges between approximately 200 and 300 mPa*s. This is roughly equivalent to the viscosity of glycerol 86 % at 20 °C.

This upper limit of viscosity is now pushed upwards by the new ViscoTip for the Multipette system. This way, even thick, sticky liquids, such as honey or creams up to 14,000 mPa*s, may now be processed precisely and quickly. The ViscoTip is a member of the family of Combitips advanced, but it can be easily distinguished, and it is automatically recognized by Multipettes.

Spring 2018, the ViscoTip was introduced to expert audiences from different industry branches for the first time, and it received substantial positive acclaim. For more detailed information, please visit www.eppendorf.com/multipette-system.



Viscosity check: ViscoTip vs. Combitips advanced. The ViscoTip expands the range of application to 14,000 mPa*s (results obtained with Multipette E3/E3x).

Even thick creams can be processed using the ViscoTip and Multipette.

Scan the QR code for more information!

TANJA MUSIOL, EPPENDORF AG

Next Generation Sequencing – Easy and Efficient

Next Generation Sequencing (NGS) has largely replaced the Sanger sequencing method. The NGS process features considerably higher efficiency and it allows sequencing of multiple samples within a shorter period of time. Eppendorf successfully introduced its solutions, with a special focus on those time-consuming steps involved in library preparation and general sample processing, at the SLAS automation exhibition in January 2018 in San Diego, California.

In-depth interaction with users

The automation trade show SLAS is a platform for the display of solutions to a broad spectrum of different applications. Over the years, Eppendorf has established itself at the SLAS as a supplier of automated liquid handling systems. This year, Eppendorf introduced special solutions on the topic of Next Generation Sequencing. A diverse team of experts manned the Eppendorf booth and took the opportunity to engage NGS users in in-depth, discipline-specific conversation. This level of advisory expertise was met with positive feedback by the visitors to the booth, and many questions on automation and NGS were answered right then and there.

Optimized automation solutions

The centerpiece of the exhibition booth was the ep*Motion*® 5075t NGS solution package: an automated Liquid Handling system, which, outfitted with new software features as well as new equipment complete with consumables, is specifically tailored to the requirements of the NGS workflow.

The automated liquid handling workstation ep*Motion* optimizes the NGS workflow.

A collaboration with one of the leading suppliers in the field of NGS kits and sequencing, the company Illumina® with headquarters in California, enabled the establishment of more than 15 individually customized methods. These methods are optimized to suit the automated use of the Illumina kits, verified by Illumina itself, and they are available as pre-established methods for the ep*Motion* 5075t to interested customers. NGS protocols thus no longer need to be established and optimized on-site. This saves both time and money.

Equipment for maximum sample yield

Besides the ep*Motion*, Eppendorf offers additional instruments and consumables that can increase the efficiency of the NGS workflow. Since the above-mentioned processes involved in library preparation are extremely time-consuming and require the completion of several cycles, each time-saving equals an increase in efficiency. As a rule, the higher the initial concentration of a sample, the fewer cycles are necessary. Sample loss or inaccurate determination of the concentration may thus negatively impact efficiency.

Eppendorf LoBind® consumables ensure that ideally no sample material is lost to adhesion to the vessel walls. The Eppendorf BioSpectrometer® fluorescence allows precise and sensitive quantification of nucleic acids, whether in the UV/Vis range or with the help of fluorescent dyes. The quick heating and cooling rates of the new Mastercycler® X50 PCR cycler ensure the highest possible enrichment of sample material while simultaneously saving time.

More information

If your curiosity is piqued and you would like to learn more about the Eppendorf solutions surrounding the topic of NGS, simply visit us at one of the upcoming exhibitions or on our website **www.eppendorf.com/ngs-made-easy**.

Scan the QR code for more information!

ULRIKE BECKEN, EPPENDORF AG BIOPROCESS CENTER, JUELICH, GERMANY

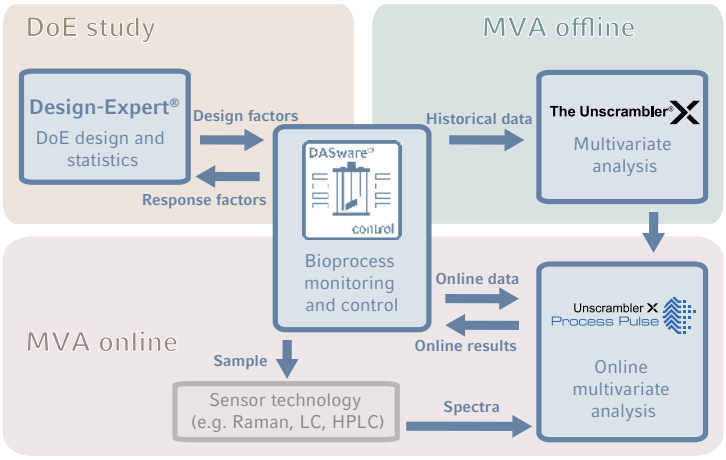
Bioprocessing Meets Statistics: Software Tools for Deeper Insight

In bioprocess development, researchers collect large amounts of data. Thorough data analysis is key to understanding how different process parameters affect the growth of cells or microorganisms and how they influence the formation and quality of the desired end product. Eppendorf and CAMO Software AS offer an integrated solution to reduce the complexity of this immense task.

Establishing robust bioprocesses

To be competitive, industrial bioprocesses need to run robustly while delivering products in consistent quality and at high yields. To establish a robust process, it is crucial to understand how variables such as the pH of the medium, the temperature, and the concentrations of oxygen, nutrients, and byproducts affect cell growth and important product characteristics. During process development, bioprocess engineers investigate these variables.

Design of Experiments (DoE) approaches help them to gather the data needed, while avoiding experiments without a benefit.



The Eppendorf DASware control 5 software and the CAMO Software packages The Unscrambler® X with Design-Expert® and Unscrambler X Process Pulse II represent an integrated software solution for bioprocess control, DoE, and MVA.

Translating data into knowledge

Using modern sensor technology and bioprocess control software, researchers can closely track multiple parameters in the course of the bioprocess, and analyze the product. However, translating the data into deeper insight is an enormous task. One difficulty, besides the sheer mass of data, is that often not only two, but many variables are interrelated.

To give an example, the formation of biomass could need sufficient concentrations of oxygen, glucose, and other nutrients in the medium, but be negatively affected by certain catabolites, whose formation is also dependent on the nutrient concentration. Identifying such complex interdependencies requires sophisticated statistical analysis.

Unlock the box

Multivariate data analysis (MVA) is the tool of choice to find patterns and relationships between several variables simultaneously. Based on this knowledge the researchers can gain comprehensive process understanding and develop advanced process control strategies. Ideally, these will allow full control: Monitoring the process in real time and taking action in case aberrations from the target state occur.

Bioprocess monitoring and control and statistical data analysis go hand in hand, but are traditionally carried out using different software tools. To close this gap, Eppendorf teamed up with CAMO, a leading supplier of software solutions for statistical analysis. The result is a seamless connection between the Eppendorf bioprocess control software DASware® control and the CAMO suite of products for MVA and DoE. This automates the transfer of data between the software packages.

In this way, the researcher can benefit from the combined capabilities of the software tools, without having to worry about the underlying data infrastructure.

Tip: Find our recorded webinar at www.eppendorf.com/webinar_mva.

Scan the QR code for more information!

CHO Cell Culture in Eppendorf BioBLU® 10c Single-Use Vessels

AMANDA SUTTLE AND MA SHA, EPPENDORF INC., ENFIELD, CT, USA

Summary

Substituting traditional glass bioreactors with single-use equipment can greatly simplify the bioprocess workflow. Single-use vessels eliminate the need for cleaning and autoclaving. This reduces the time needed to prepare the bioprocess run, and lowers the contamination risk. Another advantage, especially at larger bench scales, is the lower weight of plastic vessels compared to glass, reducing occupational hazards associated with overweight handling.

The Eppendorf BioBLU c Single-Use Vessel portfolio for cell culture covers working volumes from 100 mL to 40 L. With a working volume of 3.5 L to 10 L, the BioBLU 10c Single-Use Vessel provides an important link for bioprocess scale-up from small scale to large capacity bench scale (Fig. 1).

In this study, we tested the BioBLU 10c Single-Use Vessels in a CHO cell culture process. To evaluate process performance, we monitored cell growth and viability, the metabolic profile, and the production of an IgG antibody. We also compared the time it takes to prepare a bioprocess run using a single-use vessel and a glass vessel.

We achieved a peak cell density of approximately 12 million cells/mL, with a viability above 95 %. The results demonstrate that the BioBLU 10c Single-Use Vessel can substitute for 10 L glass vessels for larger capacity bench-scale cell culture bioprocesses.

Fig. 1: BioBLU 10c Single-Use Vessel

Material and methods

Cell line and medium

We used a proprietary suspension CHO cell line producing a human monoclonal antibody (hMAb) from TPG Biologics, Inc. We cultured the cells in CD-FortiCHO™ medium (Thermo Fisher Scientific®, USA), which we supplemented with 1x Antibiotic-Antimycotic (Thermo Fisher Scientific, USA), 1x Anti clumping agent (Thermo Fisher Scientific, USA) and 8 mM L-glutamine (complete medium).

Bioreactor inoculum preparation and inoculation

We inoculated 1×10^7 cells into a 125 mL shake flask containing 30 mL (24 % of the total volume) of pre-warmed complete medium. The CHO cells were cultivated in a New Brunswick™ S41i CO_2 Incubator Shaker (Eppendorf, Germany) at 37 °C, in an atmosphere of 8 % CO_2 and at an agitation speed of 125 rpm. We passaged the cells, without expansion, every other day for a week to allow enough time after thawing. We then expanded the culture first to three 250 mL flasks and then to six 1 L flasks. During expansion, the inoculation density, percentage fill of the shake flasks, and all other culturing parameters were kept constant. We combined the cultures from all 1 L flasks. The cell density was around 4.0×10^6 cells/mL. 99 % of the cells were viable. We used 729 mL of the combined culture to inoculate the BioBLU 10c Single-Use Vessel, containing 10 L of medium, at a targeted inoculation density of ~0.3×10^6 cells/mL.

Bioreactor control and process parameters

The BioBLU 10c Single-Use Vessel was controlled with a BioFlo® 320 bioprocess control station. We cultivated the CHO cells at 37 °C. The temperature was controlled using a heat blanket. We monitored the dissolved oxygen (DO) in the culture using an optical ISM® DO sensor (Mettler Toledo®, Switzerland), and controlled it at 50 % in 3-Gas Auto mode. Since higher gas flow can cause excessive DO fluctuation in the beginning stage and excessive foaming towards the end stage of culture, we proactively limited the oxygen flow to 0 – 3.0 SLPM and the air flow to 0.04 – 3.0 SLPM. We also added Antifoam C Emulsion (Sigma-Aldrich®, USA) as needed. We used an optical ISM pH sensor (Mettler Toledo, Switzerland) to control the pH during the run at 7.0 (deadband = 0.2), using a cascade to CO_2 (acid) and 0.45 M sodium bicarbonate (base).

We took a sample from the vessel daily and measured the cell density and viability, the pH, and the concentrations of various metabolites offline. The built-in DO and pH sleeves in the BioBLU 10c Single-Use Vessel allowed the use of optical DO and pH sensors non-invasively without the need for sensor sterilization. The non-invasive design increases the sensor lifespan, because it eliminates the incremental sensor damage caused by repeated sterilization at 121 °C.

Analytics

We measured the cell density and viability once a day using a Vi-Cell® XR viability analyzer (Beckman Coulter®, USA). We also measured the pH offline. We used these values to standardize the controller's optical pH calibration and reduce discrepancies between online and offline pH measurements.

CHO Cell Culture in Eppendorf BioBLU® 10c Single-Use Vessels

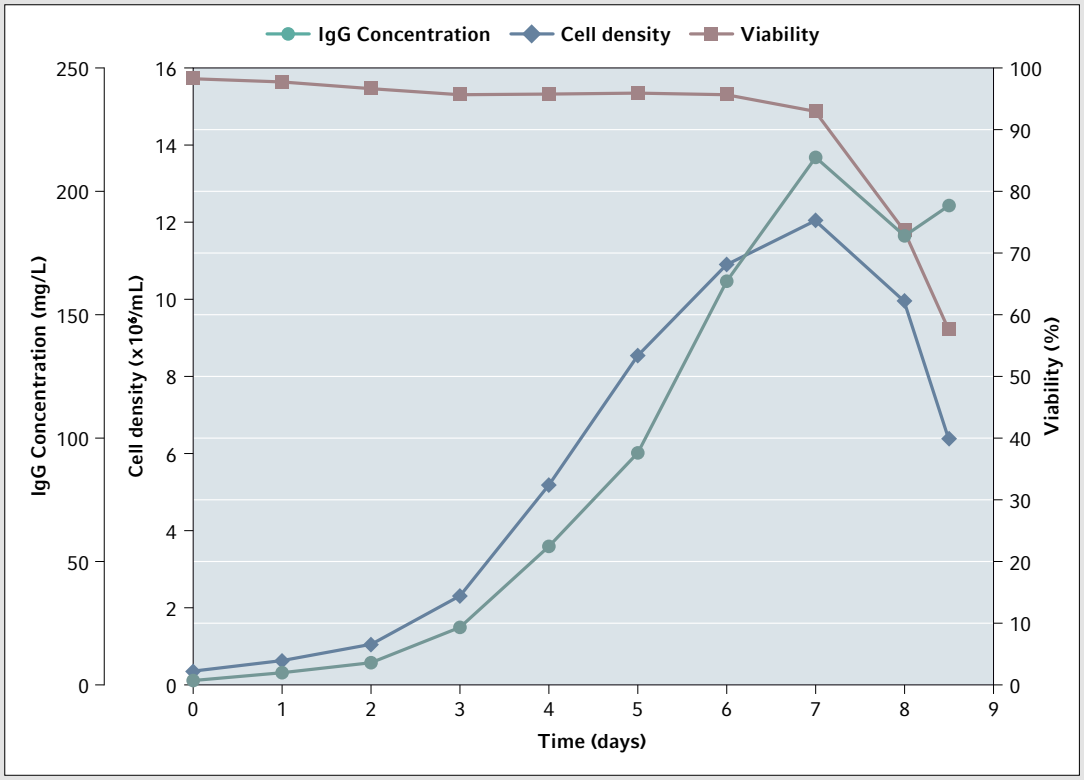


Fig. 3: Bioprocess data, measured during the CHO cell culture batch process

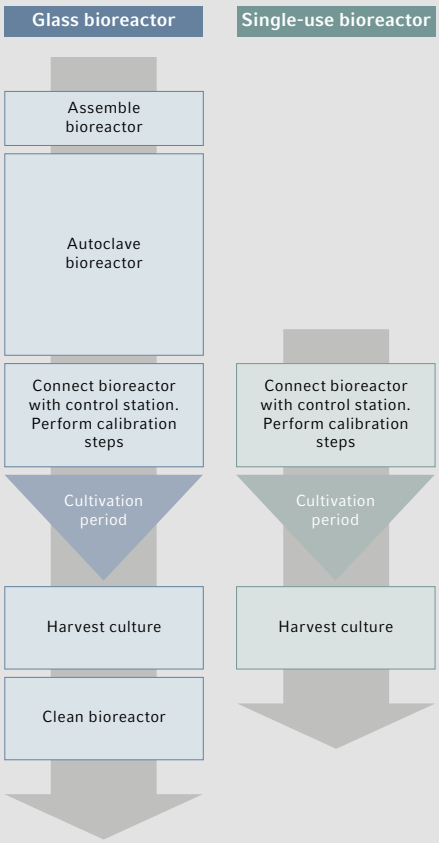


Fig. 2: Sequence of events in bioprocess runs using glass and single-use bioreactors

Results

Preparation time

Fig. 2 compares the time needed for preparation and tear-down of glass and single-use bioreactors. The use of the BioBLU 10c Single-Use Vessels saved time, because the vessel did not need to be autoclaved before the run and cleaned after (Fig. 2).

Bioprocess data

In the bioprocess run we reached a peak in viable cell density on day 7 at 12.05 x 10^6 cells/mL. After that, cell density and viability declined as anticipated (Fig. 3). The cell growth curve in the single-use bioreactor was comparable to growth of this cell line in a batch process in a traditional glass vessel [1].

By day 7, the cells had consumed the initially supplied glucose. The ammonia concentration gradually increased every day up to 10.52 mmol/L on day 8 (data not shown). Both the depletion of glucose and the rise of the ammonia concentration probably contributed to the decrease of the viable cell density, starting from day 7.

In the eight days of the batch culture, the cells had produced 212 mg/L of hMAb (Fig. 3). We terminated our culture by the end of day 8.

Conclusion

We obtained more than 12 million cells/mL in a CHO batch bioprocess. Using the BioBLU 10c saved a significant amount of time on preparation, vessel tear-down, and cleaning as compared to typical glass vessels. Furthermore, operational safety risks were reduced, due to the much lower weight of the single-use vessel.

Literature

[1] Willard S, Suttle A, Han K, Dorceus M, Cheng P-J, Sha M. Comparing culture methods for monoclonal antibody production. *BioProcess International*. 2017.

Ready-to-Use CCCadvanced™ FN1 Motifs Surface for Xeno-Free Expansion of Human Pluripotent Stem Cells

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Abstract

Pluripotent stem cells (PSCs) represent an important tool for biological research. The challenge during cultivation of PSCs is the preservation of their pluripotency. The selection of a defined culture system consisting of a growth surface and culture medium is crucial when aiming at stem cell applications requiring high consistency. Commonly used biological coating materials for PSC expansion present non-defined growth surfaces with high variability due to their biological origin and preparation using a self-coat substrate. The ready-to-use CCCadvanced FN1 motifs surface offers a comfortable alternative for PSC cultivation on a completely synthetic surface without any animal or human components. The fibronectin-derived motifs support cell attachment by mimicking native extracellular matrix proteins and allow expansion of PSCs even in restrictive xeno-free culture conditions. Thus, the FN1 motifs surface combines convenience with reliable performance: the ready-to-use consumables significantly reduce labor time and effort for scientists while offering a fully synthetic culture system with a high level of consistency during long-term PSC expansion.

Introduction

PSCs offer considerable and exciting promises in a wide range of cell applications [1]. Over the last few years, expansion conditions have progressively moved from the traditional mouse embryonic fibroblast feeder layer-based culture system towards more defined feeder-free systems [2]. But gold-standard biological coating types, for example Corning® Matrigel®, are enriched with undefined growth factors and extracellular matrix (ECM) components known to sustain cell adhesion and pluripotency. They include a non-defined composition, variable lot-to-lot quality and purity as well as a potential pathogen contamination risk.

Fully synthetic, animal-component-free culture systems representing consistent and defined culture conditions are of great interest to ensure robust cell performance in downstream applications [3]. Based on a proprietary coating technology, the CCCadvanced FN1 motifs surface is composed of synthetic fibronectin-derived motifs, specifically designed to mimic the cell attachment site of native ECM proteins. Used in combination with a well-defined culture medium and dissociation solution, this surface represents an effective animal- and human-component-free alternative to the conventional feeder layer-based culture system and to other culture systems that depend on biological coating. Here we show that the FN1 motifs surface is highly suitable for the long-term expansion of undifferentiated human induced pluripotent stem cells (hiPSCs) representing genetically modified adult cells, which exhibit a pluripotent stem cell-like state.

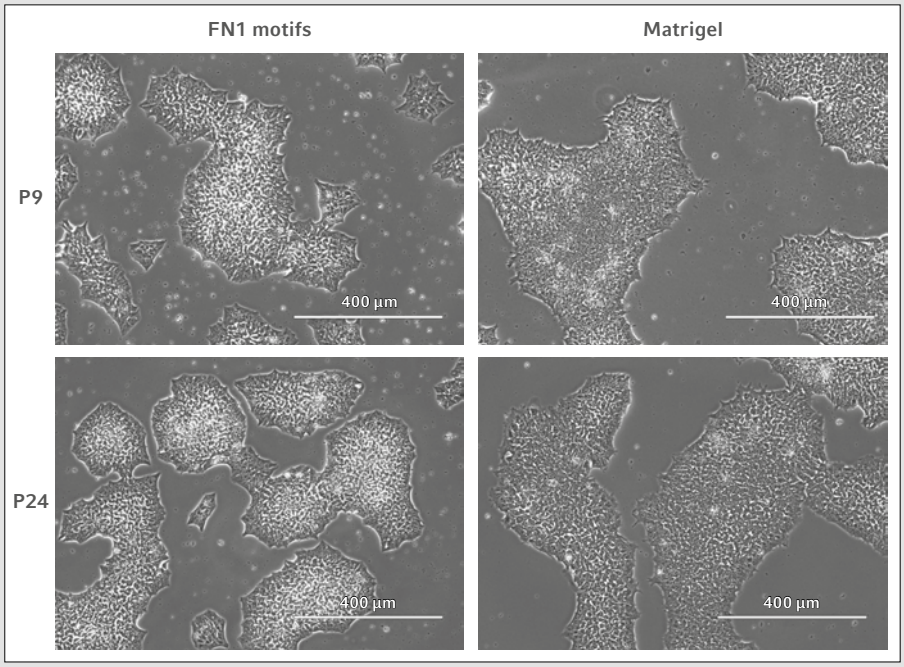

Fig. 1: hiPSCs morphology during long-term expansion

hiPSCs cultured on the FN1 motifs surface as well as on the Corning Matrigel-coated surface showed comparable homogenous flat and shiny colonies with well-defined borders. The images show representative areas at passage numbers 9 and 24, respectively. Scale bar indicates 400 µm.

Materials and methods

Cryopreserved hiPSCs were initially thawed and pre-cultivated on a Matrigel-coated surface (Corning, USA) in a xeno-free culture medium specifically adapted for feeder-free hiPSC expansion. After 5 passages, cells were seeded on the FN1 motifs surface using the classic clump passage procedure. The culture medium was replaced completely with fresh medium daily until the confluency level of interest was reached. hiPSCs were maintained on FN1 motifs up to 25 successive passages. The hiPSC growth performance on the FN1 motifs surface was compared with hiPSCs cultured in parallel on a Corning Matrigel-coated surface in a biological feeder-free culture system. hiPSCs were characterized based on their morphology, doubling time, pluripotency marker expression, karyotype, and *in vitro* trilineage differentiation potential. Detailed information about material and methods is available in Application Note 389 [4].

Results and discussion

Morphology and proliferation

The FN1 motifs surface supports a robust long-term expansion of undifferentiated hiPSCs in xeno-free conditions.

Ready-to-Use CCCadvanced™ FN1 Motifs Surface for Xeno-Free Expansion of Human Pluripotent Stem Cells

The hiPSC morphology corresponded to the cell morphology expected in a feeder-free culture system (Fig. 1) and remained stable across all 25 passages. An efficient and stable hiPSC doubling time was measured at an average value of 24 h. Morphology and doubling time were similar to hiPSCs expanded on Matrigel-coated surface.

These findings show that cells expanded on the FN1 motifs surface present morphology and proliferation rates comparable to cells expanded on the reference feeder-free culture system.

Functional pluripotency

An extensive characterization of the pluripotency phenotype confirmed the functional pluripotency of hiPSCs expanded on FN1 motifs. During the entire expansion process, very low levels of spontaneous cell differentiation were observed for hiPSCs (<2 % across the entire 6-well surface), which was confirmed by alkaline phosphatase staining. After 25 successive passages on FN1 motifs, hiPSCs expressed pluripotent specific surface proteins, TRA-1-60 and SSEA4, and self-renewal-associated nuclear transcription factor proteins, SOX2 and OCT4 (Fig. 2).

The image-based analysis was confirmed by flow cytometry analysis of the quantitative expression of key pluripotency-associated transcription factors: hiPSCs still exhibited a high level of Nanog, OCT3/4, and SOX2 expression (>95 % positive cells in the entire population), which was similar to that observed in hiPSCs expanded on the Matrigel-coated surface. The *in vitro* differentiation into cells of the three embryonic germ layers finally confirmed the functional pluripotency of hiPSCs after 20 passages on FN1 motifs surface (Fig. 3).

Genomic stability

As long-term expansion under feeder-free conditions can be responsible for the occurrence of chromosomal abnormalities in PSCs, it is very important to monitor genomic stability. A G-banding karyotype analysis performed with hiPSCs obtained after 20 successive passages on FN1 motifs in xeno-free culture medium revealed a normal human karyotype (46XY) without chromosomal abnormalities. This confirmed the genomic stability of hiPSCs after long-term expansion on the FN1 motifs surface.

Conclusion

The ready-to-use FN1 motifs surface supports long-term hiPSC expansion with a consistent growth rate of hiPSCs displaying their characteristic morphology in a completely defined culture system. During the expansion process across at least 20 successive passages, hiPSCs remain undifferentiated and retain all pluripotent stem cell-specific features, including the trilineage differentiation potential, as well as their genomic integrity.

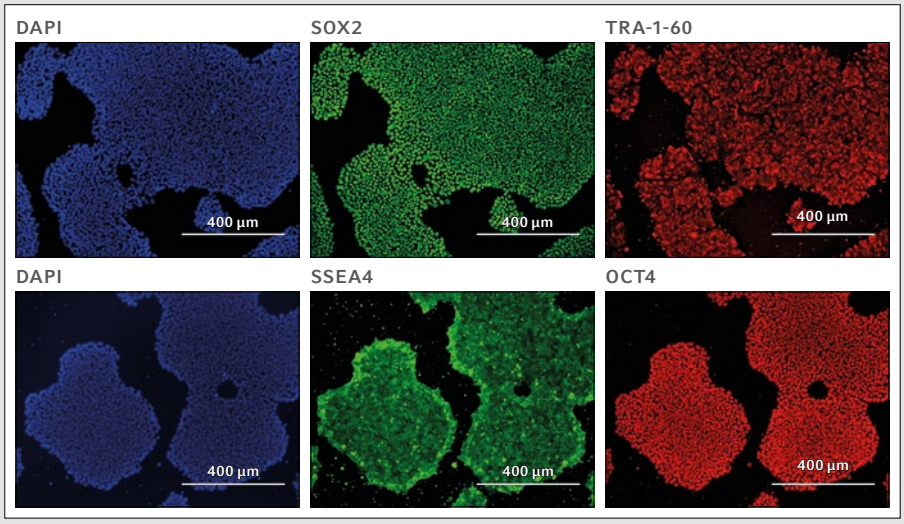

Fig. 2: Immunofluorescence staining of pluripotency markers after long-term expansion of hiPSCs

After 25 successive passages on the FN1 motifs surface, hiPSCs expressed the pluripotency markers SOX2, TRA-1-60, SSEA4 and OCT4 evaluated by immunofluorescent staining. Cells were counterstained with DAPI. The images show representative areas of hiPSCs stained after 25 passages in culture.

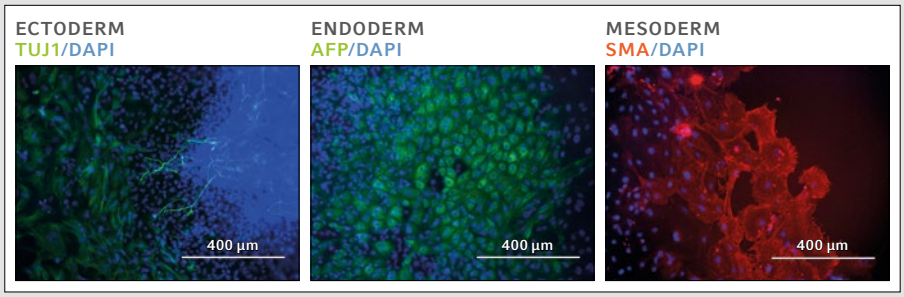

Fig. 3: Trilineage differentiation potential after long-term expansion of hiPSCs

After 20 successive passages on the FN1 motifs surface, hiPSCs maintained their trilineage differentiation potential examined by specific fluorescent staining of three specific embryonic germ layer markers: β-III tubulin (TUJ1) in ectoderm, alpha-fetoprotein (AFP) in endoderm and smooth muscle actin (SMA) in mesoderm. Cells were counterstained with DAPI.

Literature

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Scan the QR code for more information!

ViscoTip®: Optimized Performance for Highly Viscous Liquids

HANAË HENKE AND ANDREAS RATZKA, EPPENDORF AG, HAMBURG, GERMANY

Abstract

In this Application Note we measured precision and accuracy when dispensing 99.5 % glycerol using ViscoTip pipette tips and showed that both, systematic and random error, were within the ISO 8655 error limits for distilled water. Additionally, we compared 10 mL ViscoTip pipette tips and 10 mL Combitips advanced® in handling various viscous liquids.

Combitips advanced could handle liquids with a viscosity of up to 200 mPa*s (e.g. ~86 % glycerol). In contrast, ViscoTip pipette tips could also handle highly viscous liquids (e.g. liquid honey) easily, and aspiration forces as well as dispensing forces were reduced.

In summary, we recommend using the ViscoTip for reliable handling of viscous liquids with a viscosity of > 200 mPa*s.

Introduction

Highly viscous liquids such as glycerol or Tween® 20 are commonly used in laboratories – often as components of buffers. Due to their special flow behavior, these liquids often lead to difficulties in handling. Positive displacement devices, working like a syringe, offer easier handling of viscous solutions up to their individual limit. But at a certain degree of viscosity, precision and accuracy generally decrease while the aspiration and dispensing forces increase.

Viscosity is determined by different types of viscosimeters, most of them measuring the liquid movement, called dynamic viscosity. Dynamic viscosity is determined as the force needed by a liquid to overcome its own internal molecular friction to flow. It is expressed in the measurement unit mPa*s (milli Pascal second) [1, 2].

In this Application Note we tested and compared ViscoTip pipette tips and Combitips advanced in terms of precision, accuracy, and forces needed for aspiration and dispensing.

Materials and methods

Determination of systematic and random error with 99.5 % glycerol

Both the systematic and random errors of ViscoTip pipette tips were determined gravimetrically using 99.5 % anhydrous glycerol. Measurements were performed according to ISO 8655 standards. Since error limits for glycerol do not exist, the limits for distilled water stated in ISO 8655 [3] and the stricter Eppendorf error limits of 10 mL Combitips advanced [4] were used as references.

Determination of correct dispensing volumes using various viscous liquids

The correct dispensing volume of 10 mL Combitips advanced and 10 mL ViscoTip pipette tips was determined gravimetrically using various viscous liquids. Multipette® E3x (U.S./CAN: Repeater® E3x) was used for testing. Speed levels 5 or 2 were selected for aspiration and dispensing, respectively.

Comparison of aspiration and dispensing forces of various viscous liquids

Measurements of aspiration and dispensing forces expended when pipetting liquids with Combitips advanced and ViscoTip pipette tips, respectively, were carried out using the digital force gauge FH 100 (Sauter GmbH, Germany). 5 mL of each liquid were aspirated and dispensed five times. Then the arithmetic mean was calculated.

Results and discussion

Systematic and random errors obtained with 99.5 % glycerol are within ISO limits for distilled water

Using classic dispensing tips, 99.5 % glycerol is among the most frequent and yet highly challenging liquids in the lab. In our test series, the systematic and random error using Multipette/Repeater M4 with ViscoTip pipette tips for dispensing 99.5 % glycerol were well within the ISO 8655 error limits defined for distilled water. Furthermore, errors were also within the much stricter Eppendorf error limits for positive displacement dispensers.

When using a Multipette/Repeater E3x with ViscoTip pipette tips, the error limits complied with ISO 8655 for distilled water (Table 1).

Multipette®/Repeater® E3x	Measured values			ISO 8655 error limits		
	1,000 µL	5 mL	10 mL	1,000 µL	5 mL	10 mL
Systematic error [%]	−0.3	−0.3	−0.3	±5.0	±1.0	±0.5
Random error [%]	0.3	0.2	0.1	±3.0	±0.6	±0.3

Multipette®/Repeater® M4	Measured values			ISO 8655 error limits		
	200 µL	1,000 µL	2,000 µL	200 µL	1,000 µL	2,000 µL
Systematic error [%]	−0.3	−0.3	−0.3	±8.0	±1.6	±0.8
Random error [%]	0.5	0.3	0.2	±4.0	±0.8	±0.4

Table 1: Systematic and random error of ViscoTip using 99.5 % glycerol (n = 10)

ViscoTip®: Optimized Performance for Highly Viscous Liquids

Liquid	Dynamic viscosity in mPa*s	Combitip advanced 10 mL	ViscoTip® 10 mL
Mineral oil	26 (23°C)	√	√
Motor oil	130 (23°C)	√	√
Paraffinum oil	200 (20°C)	√	√
Triton® X-100	240 (25°C)	(√)	√
Tween® 20	400 (25°C)	(√)	√
Viscosity standard APN1400	5,041 (20°C) 3,418 (25°C)	X	√
Liquid honey	12,500 (22°C)	X	√
Hand cream	13,600 (23°C)	X	√
Viscosity standard APN4000	14,042 (20°C) 9,256 (25°C)	X	√

Table 2: Comparison of pipettability for various viscous liquids using Combitips advanced and ViscoTip
√ Dispensed liquid volumes were correct;
(√) Error message in the last dispensing step;
X Dispensed liquid volumes were incorrect or aspiration/dispensing impossible

In conclusion, the results show that ViscoTip is as precise and accurate when dispensing 99.5 % glycerol as when using other direct displacement tips with distilled water.

The ViscoTip outperforms classic tips using various viscous liquids

Using 10 mL Combitips advanced with various viscous liquids, it was shown that a limit in handling is reached at a dynamic viscosity around 200 mPa*s. Liquids with a higher viscosity cannot be dispensed precisely past this point (Table 2).

In contrast, ViscoTip pipette tips used for dispensing various viscous liquids enable dispensing of the correct liquid volume. Each tip was pre-wetted prior to the measurement. Our tests showed that pre-wetting is essential to reach reliable and repeatable results using ViscoTip pipette tips. We were able to show that a broad range of liquids could be handled using ViscoTip pipette tips, ranging from <200 to 14,000 mPa*s. In comparison, Combitips advanced 10 mL generally showed a limit of handling viscosity at approx. 200 mPa*s. For Tween 20 and Triton® X-100, the last dispensing step using Combitips advanced was incorrect or led to an error message. In contrast, all tested liquids could be aspirated and dispensed using the ViscoTip.

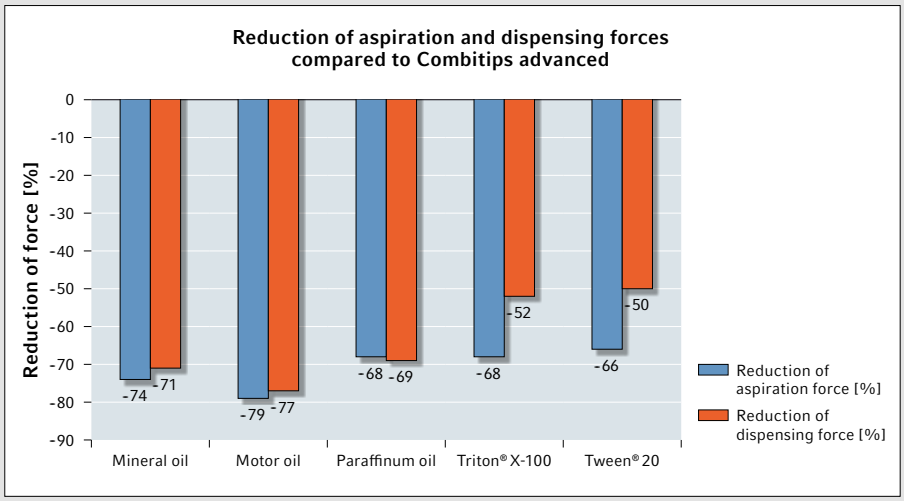

Fig. 1: Reduction of forces when aspirating/dispensing liquids using ViscoTip compared to Combitips advanced (baseline). n=3

The ViscoTip reduces aspiration and dispensing forces

Another important parameter when pipetting viscous liquids is the operating force necessary to aspirate and dispense the liquid.

The liquids that showed correct dispensing volumes in the previous test were also used to determine operating forces with Combitips advanced. Using ViscoTip pipette tips, it was shown that aspiration and dispensing forces were highly reduced in comparison to Combitips advanced (Fig. 1).

Thus, we recommend using ViscoTip pipette tips even for liquids that could be pipetted with both tip variants in order to achieve the best results with least effort.

Conclusion

The results of testing various viscous liquids showed that the ViscoTip could handle viscous liquids up to 14,000 mPa*s. Furthermore, it complied with the error limits stated by ISO 8655 and the stricter Eppendorf error limits when dispensing 99.5 % glycerol. Additionally, aspiration and dispensing forces were highly reduced when using ViscoTip pipette tips compared to Combitips advanced.

In general, we recommend using 10 mL Combitips advanced for liquids with a maximum viscosity of 200 mPa*s. For liquids of higher viscosity, ViscoTip pipette tips should be used to maintain correct dispensing results.

Literature

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[4] Standard Operating Procedure. www.eppendorf.com/sop

The 10 µL Dispensing Tool Ensures Highly Accurate and Precise Sub-Microliter Volume Pipetting on ep*Motion*® 5073l

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DOMINIK SCHNEIDER, EPPENDORF AG, HAMBURG, GERMANY

Abstract

In this report we show the possibility of automating a quantitative Polymerase Chain Reaction (qPCR) setup involving small reaction volumes by using the ep*Motion* 10 µL dispensing tool. For PCR setup, a sample volume of 0.2 µL was used in a total reaction volume of 5 µL. The qPCR performance was evaluated by a highly sensitive qPCR assay. It was demonstrated that a low volume qPCR setup can be successfully automated on the ep*Motion* 5073l, as excellent accuracy and reproducibility were obtained. The use of the ep*Motion* workstation increases the consistency and efficiency of small volume dispensing by removing the human and day-to-day variability. In addition, researchers will save time and money by eliminating repetitive work and by reducing the use of costly reagents, respectively.

Introduction

Nowadays, laboratory processes are becoming more and more complex, driving the need for assay miniaturization. The tendency towards assay miniaturization is present in a lot of different applications, like compound screening for drug discovery in the pharmaceutical industry [1].

Indeed, the ability to perform primary screening assays in high-density microwell plates at volumes of 1–2 µL will accelerate the early stages of drug discovery and reduce costs. Another field is in molecular biological research using the quantitative PCR technology. However, qPCR is an expensive technology. One way to decrease the costs is to reduce reagent volumes.

Assay miniaturization offers further advantages, as the volume of extracted DNA available for qPCR is limited in many applications such as forensic analysis [2].

A miniaturized assay allows to perform qPCR even with a low amount of DNA or to run more experiments with the same amount of biological sample leading to better result interpretation. Downscaling also increases the number of reactions performed in parallel and reduces the analysis time.

Therefore low throughput qPCR systems are more and more replaced by 384-well instruments. Despite the advantages miniaturization asks for dispensing small liquid volumes accurately and precisely. Delivery of sub-microliter volumes is difficult to achieve and is a major obstacle for the implementation of miniaturized assays. One of the issues that all labs are facing is the human error as miniaturized assays are typically performed in 384-well plate formats. Therefore the smaller the dispensing volume, the greater the operator's expertise should be. Lastly, beside the human factor, environmental conditions such as small variations in laboratory temperature and humidity can have a significant effect on the correct handling of small volumes. With so many variables affecting the dispensing process, choosing the proper solution for small volume handling is of the highest importance. One solution, especially when a large sample number is required to be processed in a short time, is automation. The capability of the ep*Motion* liquid handling workstation to automate a qPCR assay was already demonstrated [3-5].

By reducing human intervention and thanks to an accurate pipetting system, ep*Motion* automated liquid handling systems provide high assay reproducibility without cross-contamination, ensuring reliable results.

The new 10 µL dispensing tool developed for the ep*Motion* automated liquid handling systems allows accurate and precise dispensing of volumes as low as 200 nL. The purpose of this Application Note is to demonstrate the efficiency of the 10 µL tool in a qPCR setup by using small sample volumes.

Left: 10 µL ep*Motion* dispensing tool single-channel used for this Application Note (also available as 8-channel tool, shown right)

Materials and methods

The KAPA® Library quantification kit for Illumina® sequencing platforms was used in the experiments. The kit contains Master Mix, primers (sense: 5'-AAT GAT ACG GCG ACC ACC GA-3'; anti-sense: 5'-CAA GCA GAA GAC GGC ATA CGA-3') and ready-to-use DNA standards. Each reaction was carried out in a total volume of 5 µL containing 4.8 µL of Master Mix with primers and water. As template DNA 0.2 µL of DNA standards were used. The mix was subjected to the following thermal conditions: 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s and 60 °C for 45 s.

The 10 µL Dispensing Tool Ensures Highly Accurate and Precise Sub-Microliter Volume Pipetting on ep*Motion*® 5073l

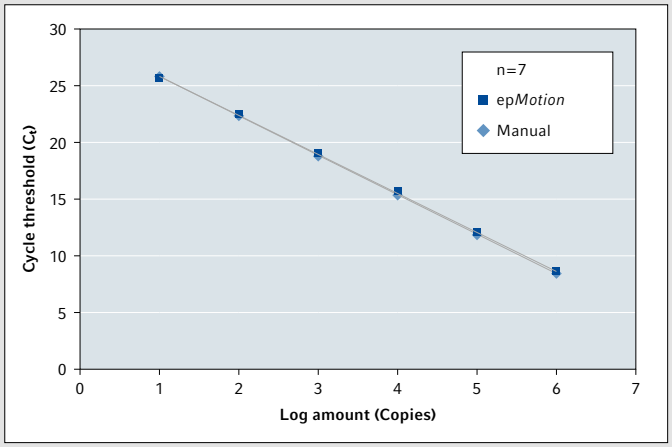

Fig. 1: Standard curves generated for KAPA Library Quantification kit. Standard curves were generated using six DNA standards with template volumes of 0.2 µL

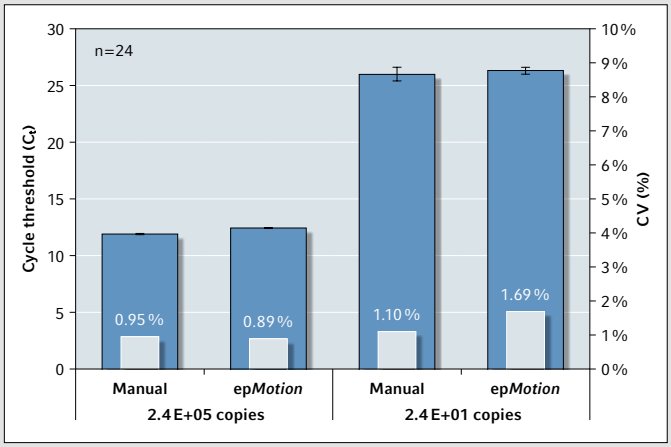

Fig. 2: Reproducibility of KAPA Library Quantification kit. Mean inter-sample C_t value, standard deviation and coefficient of variation (CV) were calculated for each plate containing 24 positive samples.

Automated PCR preparation was performed on an ep*Motion* 5073l system. 4.8 µL of the final Master Mix including the oligonucleotides was dispensed into a PCR 96-well plate followed by the addition of 0.2 µL DNA template or H_2O for non-template controls.

Results and discussion

The automation of the qPCR setup using the 10 µL dispensing tool and the ep*Motion* 5073l was compared to manual setup. For a direct comparison, the manual and automated setups were performed on the same day using the same qPCR plate.

Assay efficiency and reproducibility

To evaluate the dispensing efficiency of the ep*Motion* 5073l a very sensitive qPCR assay, the KAPA Library Quantification kit, has been selected. The kit provides all reagents needed for the quantification of Illumina libraries by qPCR as well as six ready-to-use and well-defined standards. Efficiencies were calculated based on a standard curve of the DNA standards and showed comparable results to manual PCR preparation (Fig. 1, Table 1).

Parameters	Manual	ep*Motion*
Slope	-3.48	-3.42
Efficiency	94 %	96 %
R^2	1.0	0.9995
Detection limit	24 copies	24 copies

Table 1: qPCR efficiency parameters for KAPA Library Quantification kit

The assay reproducibility was evaluated by comparing results generated from 24 positive samples containing a low and high number of DNA copies per qPCR reaction. It could be shown that the mean C_t value is very consistent for a defined amount of target for the automated setup as well as for the manual setup. The coefficient of variation of the automated qPCR setup does not exceed 0.89 % for high copy numbers and 1.69 % for low copy numbers (Fig. 2).

Conclusion

In this Application Note, the capability of the ep*Motion* 5073l liquid handling system to automate a complete qPCR assay setup involving very low volumes such as 0.2 µL was demonstrated.

Results obtained with the automated qPCR setup were highly accurate and reproducible, yielding data similar to a manual preparation. As a highly sensitive application, qPCR was used to demonstrate the robustness of the 10 µL ep*Motion* dispensing tool. Dispensing of sub-µL volumes can be extended to a large variety of applications requiring handling of small volumes such as protein applications, compound screening, or cell-based assays.

The presented results clearly indicate that the automated ep*Motion* equipped with the 10 µL dispensing tool provides a high assay reproducibility ensuring reliable results by reducing human error and by providing an accurate pipetting performance.

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MATTHEW JURKIEWICZ, EPPENDORF AG

Innova® S44i: Grow More Cells in Less Space

Eppendorf and Innova Biological Shakers have been helping scientists achieve better results for over 70 years. Now, it's time to move labs forward by moving shaker technology forward – to grow labs by growing better cultures. Learn more about the new Innova S44i Biological Shaker, the next great addition to a renowned portfolio of life science solutions. Reap the benefits of its performance and reliability and expand the potential of your laboratory!

S44i: high capacity stackable shaker

Space in the lab is always at a premium. Every device needs to produce maximum results in relation to its footprint and size. The Innova S44i has an optimized chamber, platforms, and features for enhanced options and capacity. The large chamber easily accommodates Erlenmeyer flasks up to 5 L and a wide variety of racks, plates, and vessels for flexibility. It can be double- or triple-stacked to increase capacity without increasing its footprint. Within the chamber, an optional static shelf expands capacity for incubating samples that do not require agitation.

Innova S44i: the next generation in biological shakers

Reliable shaking

Since its introduction, the cast iron triple-eccentric drive in previous Innova Shakers has been the gold-standard in shaker drive technology. It is the foundation upon which Innova Shakers and the trust of the scientific community are built. Now, the new, improved, and patented* Eppendorf X-Drive has been designed to offer even greater performance, flexibility, and peace-of-mind for the modern biological shaker. The Eppendorf X-Drive is precisely engineered with five-eccentric shafts to balance even the heaviest loads while providing smooth and consistent shaking. The robust and industrial construction of the drive is designed for round-the-clock operation (*Patent number US 8,226,291 B2).

Touch the future

But all this capacity and reliability is wasted if scientists cannot easily set their programs, monitor their progress, and export their results. The new touchscreen user interface of the Innova S44i offers more than just easy operation. This multi-touch capacitive display makes all the information easy to monitor and track. It is all just one touch away with a customizable home screen, comprehensive data displays, multi-step programs, and user authorization management.

VisioNize®: labwork meets network

With the addition of the VisioNize system to the Innova S44i, labwork meets network.

With VisioNize, labwork meets network.

VisioNize easily links lab equipment to central monitoring and data management software. It's a smart network that organizes devices efficiently whenever and wherever needed. The Innova S44i is a VisioNize-onboard device that connects directly to "VisioNize core" and can be remotely monitored from a PC or on-the-go with the "VisioNize go" mobile app.

Learn more about the next evolution in biological shakers, the Innova S44i: www.eppendorf.com/InnovaS44i.

CHRISTIAN HABERLANDT, EPPENDORF AG

CellXpert® C170i: New Specialist for the Cultivation of Sensitive Cells

Sensitive cells such as stem cells or primary cells place high demands on their culture conditions. Key factors include the right cell culture medium, an optimal growth surface, and a highly stable atmosphere inside the incubator. The new Eppendorf CellXpert C170i sets new standards in CO_2 incubation. It ensures high flexibility for the future, it simplifies comprehensive, fail-safe documentation, and it is an integral component of the networked laboratory of tomorrow.

Secure retrieval of vessels holding valuable cells and media. For example, inner and outer doors may be closed using your elbow only.

Flexibility for the future

Are you able to decide today which duties your new incubator will need to take on five years from now? Which cells you will be working with, and under what conditions? Can you predict staffing and space allocations in the laboratory? The CellXpert C170i allows you to adapt to changing demands and conditions in a flexible manner. To this end, besides many factory standard options, a variety of future upgrades are available to you, directly in your lab. For instance, if you decide two years from now that you want to culture stem cells under hypoxic conditions, you will be able to simply add on an O_2 control. Even on the topic of digital transformation in the laboratory and networking, the C170i has already arrived in the future. For example, its integrated VisioNize® interface enables remote monitoring and easy documentation of incubation parameters and events (more on VisioNize on page 11).

Easy and secure documentation

The CellXpert C170i documents the culture conditions inside the chamber, events such as door openings and alarms securely on an integrated SSD drive. The data can be exported quickly and easily via USB port and filtered if required. The data, available in common data formats, are then easily stored and printed. Protocols and documentation for the 180 °C high temperature disinfection can be saved and printed quickly.

How to save time and money

Prior to the purchase of a new CO_2-Incubator, it is worth considering the following questions and comparing the answers for your incubator candidates:

> How much space is available in your lab?

> How many vessels can be optimally accommodated in your incubator?

> What is the cost of regularly exchanging either HEPA-filter or UV-lamp in your current incubator over several years?

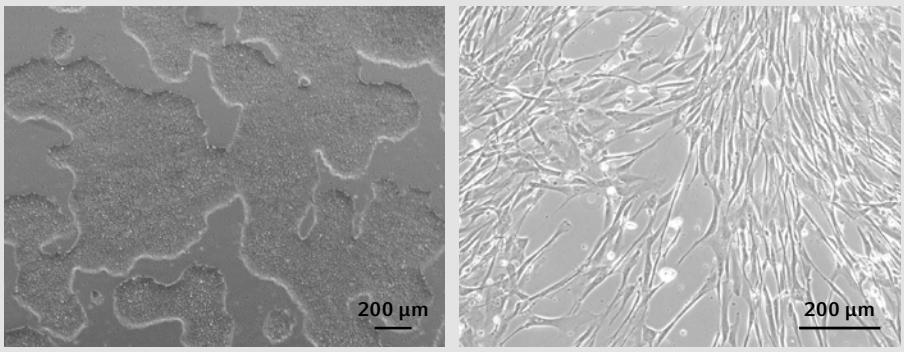
> How time-consuming is the cleaning process and how long does it take to remove internal parts for decontamination?

> How complex is the documentation of culture conditions and decontamination?

> How much gas is the device consuming?

We are sure that the answers to these questions will help you to make the right purchasing decision!

Get more info during the next visit of your Eppendorf representative.



Sensitive cells such as hiPSCs (human induced pluripotent stem cells, left) or human MSCs (mesenchymal stem cells, right) will find ideal growth conditions inside the C170i and on the new CCCadvanced™ FN1 motifs surface*.

*please also see Application Notes, pages 3–4

BERRIT HOFF, EPPENDORF AG

PCR Optimization in a New Dimension

The new Mastercycler® X50 is the elegant synthesis of speed, flexibility, and PCR optimization for research PCR applications and standardization for routine PCR applications such as food testing. With the new highly intuitive touch screen you have all these benefits at your fingertips.

Speed and enhanced PCR optimization functions like the 2D-Gradient make the Mastercycler X50 the ideal PCR cycler for advanced research in molecular biology. The excellent block temperature regulation gives rise to the next stage of PCR reproducibility, whereas the adaptable user management and profound documentation capabilities give peace of mind to laboratories working to set standards.

The intuitive touch screen interface for quick programming, low noise levels, low power consumption, and the versatile flexlid® concept complete the product to be a powerful PCR cycler. Up to 10 PCR cyclers can be combined – ideal for high throughput applications or labs with a high number of users running different assays. If you need more flexibility or higher throughput, up to 50 PCR cyclers can be combined in a computer-controlled network.

The Mastercycler X50 can, of course, also be linked to the VisioNize® system.

The next stage of PCR optimization

The innovative 2D-Gradient allows you to optimize both the denaturation temperature and the annealing temperature during the same run. Higher denaturation temperatures can have the advantage of increased specificity while lower denaturation temperatures reduce stress on biomolecules and can lead to increased yield*.

Assays that struggle to work reliably at a 95°C denaturation temperature could benefit significantly from the optimization of the denaturation temperature.

(*see Application Note 387, download at www.eppendorf.com/appnote387)

Find more information at www.eppendorf.com/mastercycler

Scan the QR code for more information!

Tip

VisioNize®: a Smart Network to Manage Your Lab

With Eppendorf's VisioNize system you can easily link your lab devices to a central monitoring and data management application. The VisioNize system consists of software applications, VisioNize-onboard devices (already equipped with VisioNize functionalities), and the VisioNize box, an easy-to-use hardware integration interface to connect additional devices. Connect your lab equipment to VisioNize and remotely monitor instruments from your office PC or with the mobile app VisioNize go.

Use the VisioNize system to optimize essential lab routines!

The software applications VisioNize core and VisioNize go allow you to

- check and monitor the performance and status of your connected lab devices
- plan downtimes for maintenance services in advance
- monitor overnight processes from home or the remaining runtime of experiments from your office
- receive relevant information and stay informed thanks to e-mail notification.

With VisioNize you can effectively organize your lab workflows and thus increase efficiency!

More information at www.eppendorf.com/visionize

SAMIRA SCHROEDER, EPPENDORF AG

Proper Handling of Cells in the Laboratory (IV)

After reporting on the topics "Prevention of contamination in cell culture", "Cell identity", and "Reproducibility of results" in previous issues of BioNews, we are now shining a light on the major consequences of mycoplasma contamination. We will show you how you can detect and, more importantly, prevent mycoplasma contamination.

The challenge

Mycoplasma are parasites with an average size of 0.1–0.3 µm that live on the membranes of cells (Fig. 1).

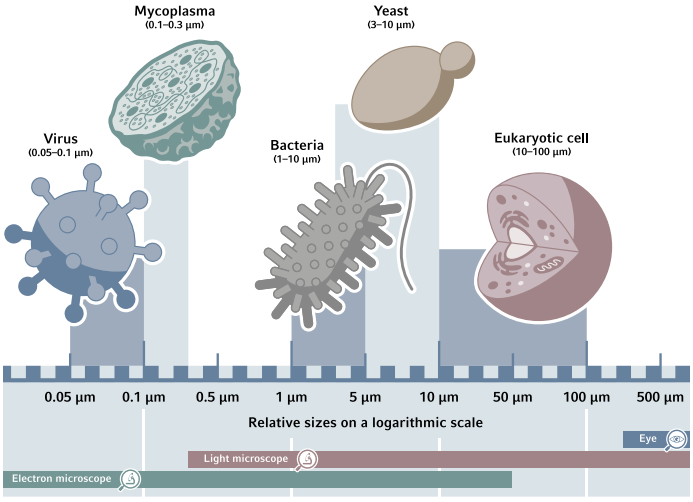


Fig. 1: Different microorganisms and their sizes, including mycoplasma which cannot be detected via bright-field microscopy.

In contrast to many other microorganisms, mycoplasma cannot be detected during routine inspection using bright-field microscopy. Their lack of a cell wall, together with relatively high plasticity, further allows mycoplasma to pass through standard membrane filters with a pore size of 0.2 µm.

Even visible changes within the medium, such as color changes or turbidity, cannot be expected with mycoplasma contaminations. These otherwise reliable indicators of microbial contamination are bound to fail in this case. The use of antibiotics can further convey a false sense of security, thus allowing the spread of mycoplasma to go unnoticed.

Consequences

Mycoplasma produce toxins and they compete with their host cells for available nutrients. The resulting influence on gene expression has the potential to impact every parameter within the host cell system and thus lead to non-reproducible data.

Prevention

Laboratory workers themselves represent the main source of mycoplasma. Especially if Good Laboratory Practices are disregarded, mycoplasma are easily introduced into any cell culture. In addition, a contamination may be transferred from one laboratory to another and remain unnoticed, for example during the exchange of cells.

According to estimates in the literature, approximately 5–30 % of all cell lines are contaminated with mycoplasma worldwide. Laboratories that subject their cells to regular testing significantly reduce their contamination rate. To improve the reproducibility of results, it is recommended that all cell cultures be tested for mycoplasma on a regular basis, using either PCR, ELISA, or microbiological culture methods.

Peace of mind with Eppendorf Cell Culture Flasks

Effective protection from contamination, along with optimum gas exchange, is provided by the innovative air filter technology of the Eppendorf Cell Culture Flasks (Fig. 2). The highly efficient volume filter prevents particles from entering the cell culture flask.

Fig. 2: Section across the highly efficient volume filter of the Eppendorf Cell Culture Flasks

Two core features of the Eppendorf filter are key to its effectiveness: Unlike other relatively thin membrane filters, the Eppendorf filter with its thickness of approximately 1 mm presents a significantly longer distance for external particles to overcome. In addition, an undefined, irregular filter structure – instead of defined pores – ensures that particles will become trapped inside the filter.

Scan the QR code for more information!

A list of mycoplasma detection methods and additional information on the topic of contamination prevention are available at www.eppendorf.com/cellexperts.

HANAË HENKE, EPPENDORF AG

Premium Source of Laboratory Expertise

Day by day, scientists are giving their best to master the challenges of their lab routine and to improve their processes. Today however, additional routine tasks and administrative duties typically compound laboratory work. As a result, there is little leeway for solution-oriented thinking or creative optimization of experiments.

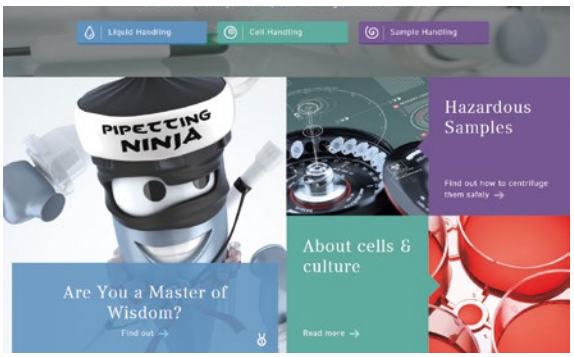
These days, when there is no time for problem-solving, we search for information that is instantly available to us. We have become used to going online at any time, via our PC, tablet, or phone. Given the vast number of forums and free online libraries, it is not always immediately obvious whether the information thus obtained is trustworthy and accurate.

For example, countless sources and references exist, which offer tips and tricks on improving pipetting techniques. Now, is one to compare them all and reach a consensus based on those items most frequently mentioned? It is easier, safer, and quicker to trust the kind of support and background information that has been compiled and verified by scientists.

Expert knowledge in an attractive package

On the Eppendorf Handling Solutions homepage www.eppendorf.com/handling-solutions you will find articles on important aspects of everyday laboratory work in the areas of Liquid Handling, Cell Handling, and Sample Handling – high-quality contributions, written by application specialists with many years of experience in the laboratory. Whether the conversion of *g*-force to rpm during centrifugation or clear information on the prevention of bacterial contamination in cell culture are required, or whether sophisticated applications and their optimization in the field of bioprocessing are concerned: It is our goal to provide you with current expert knowledge and thus simplify your daily work. Besides articles on scientific topics and application support for protocols, you will also find videos and cartoons, an entertaining quiz-section, and much more!

Our recommendation: If you are looking for easily understandable content that is closely aligned with current laboratory practice, please visit www.eppendorf.com/handling-solutions.



Scan the QR code for more information!

Tip

Top 5 Reasons to Automate NGS Library Prep

Next-generation sequencing sample preparation is a labor-intensive process, which requires experience, precision, and accuracy to generate high-quality NGS libraries. Automating NGS library preparation using the ep*Motion*® minimizes sources of error, provides reproducible results and increases your productivity even for runs with low sample numbers.

Here are the top 5 reasons for automating NGS library preparation:

Reason 1: Minimize sources of error! Because NGS reagents are very expensive, making mistakes will cost you a lot of money. Since an NGS method for 24 samples can have more than 1,200 pipetting steps, errors caused by manual pipetting cannot be excluded in such long workflows. With the ep*Motion* automated liquid handling system you minimize human intervention.

Reason 2: Improve your results! A better pipetting accuracy leads to better and more reproducible results.

Reason 3: Minimize reagent usage! A better pipetting accuracy allows reducing pipetting volumes.

Reason 4: Free up your technicians' time! Since ep*Motion* does the hard work, users don't have to!

Reason 5: Automated pipetting protects users from repetitive strain injury (RSI).

More information at www.eppendorf.com/ngs-made-easy

Scan the QR code for more information!

CAROLYN TAUBERT AND BERRIT HOFF, EPPENDORF AG

Eppendorf Prize Winners 2017/2018: Flavio Donato & Andrea Ablasser

Flavio Donato

The Italian scientist Flavio Donato, Ph.D. has won the 2017 *Eppendorf & Science Prize for Neurobiology* of 25,000 USD for his work on the driving forces that orchestrate the maturation of circuits representing space in the brain.

Dr. Donato carried out his research in the laboratory of Professors May-Britt Moser and Edvard Moser at the Kavli Institute of the Norwegian University of Science and Technology in Trondheim. Flavio Donato's work has revealed that, during development, stellate cells in the medial entorhinal cortex are the source of an activity-dependent instructive signal necessary for the maturation of those neurons that give us a sense of where we are. This finding proves the existence of autonomous, intrinsic drivers that guide the maturation of widespread regions of cortex from deep within the brain. Unravelling the unique contribution of these specific neuronal populations to the function of neural circuits will advance our understanding of how the brain processes abstract cognitive functions.

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

Andrea Ablasser

The 2018 *Eppendorf Award for Young European Investigators*, endowed with € 20,000, went to Dr. Andrea Ablasser, Assistant Professor at the Swiss Federal Institute of Technology, Lausanne, Switzerland. The award was given for her contributions to a key step in the innate immune response, which triggers a frontline defense when cells are attacked by microorganisms.

Her work sheds light on the mechanisms by which other cells are informed about the presence of foreign DNA, and she has recently shown that the same pathway can be triggered in ageing cells, contributing to senescence. "Andrea Ablasser's work may lead to novel therapeutic approaches for both microbial infections and autoinflammatory syndromes", the jury chaired by Prof. Reinhard Jahn (Max Planck Institute for Biophysical Chemistry, Göttingen, Germany) concluded.

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

Both prize winners will visit Eppendorf in Hamburg during 2018. Check out the next BioNews issue for more info!

Win 1 of 5 Personalized Pipettes

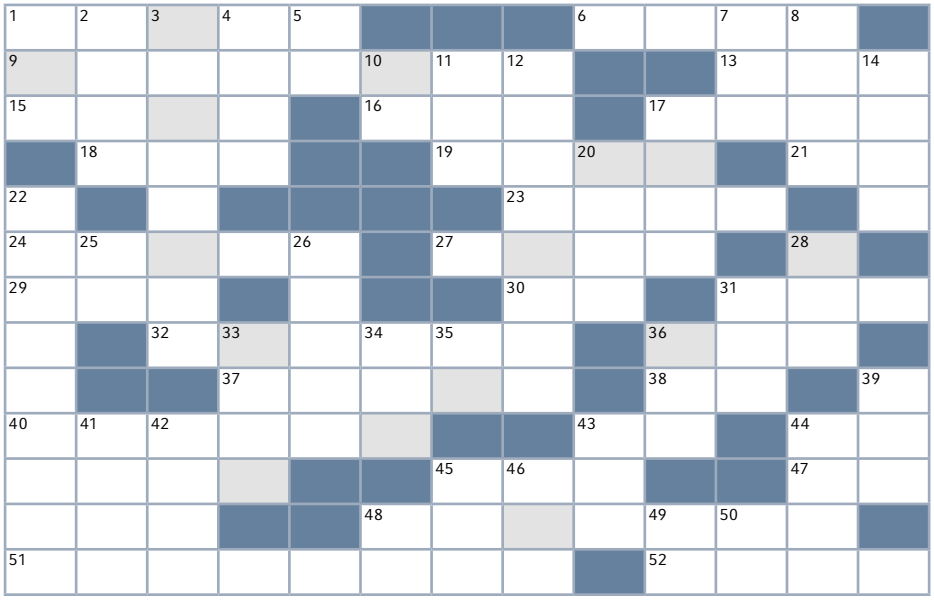
The solution of the prize competition of BioNews No. 47 was "Smart. Connected. Nize". Matthew Sullivan (University of Auckland, New Zealand) won the first prize.

Good luck in another anniversary 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, 2018**.

You can either send 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. 51.



1st to 5th Prize:

1 personalized Eppendorf Research® plus pipette (adjustable, single-channel) of your choice

6th to 10th Prize:

1 Amazon® Voucher worth 50.00 EUR

11th to 15th Prize:

400 bonus epPoints® each

(epPoints registration required)

ACROSS

1 Male given name
6 Pull apart or into pieces
9 Training in boxing
13 Large amount of something
15 International Air Transport Association (abbrev.)
16 Preposition
17 Celestial body
18 Light-emitting diode (abbrev.)
19 Move to and from, wag
21 ISO country code for Germany
23 Figurine, puppet
24 Pieces of potatoes served hot
27 Tablet, capsule
29 Hot drink
30 And (Latin)
31 Possible with in or out
32 Capital of the United Kingdom
36 Completes Paulo
37 To coat or cover
38 Original poster (abbrev.)
40 Flora and fauna
43 Long-playing record (abbrev.)
44 Polypropylene (abbrev.)
45 Side sheltered from the wind
47 Instant message
48 Famous R&B singer
51 Round worm
52 Martial science fiction adventure in conjunction with 17 across

DOWN

1 Work-related musculoskeletal disorder (abbrev.)
2 Mineral and gemstone
3 Substance of which a thing is or can be made
4 County and city in Romania
5 ISO country code for Nauru
7 Key on computer keyboard
8 Route, way
10 In the event that
11 At once, immediately
12 Function in a thermal cycler
14 Complements 17 across to an epic interstellar trip
17 Exchange for money
20 Unit of electric potental
22 Part of day
25 Chemical symbol for rhenium
26 Echo sounder
28 Garden where animals are housed for exhibition
31 One time around a course
33 Work of art
34 To pass away
35 Preposition
36 Standard Operating Procedure (abbrev.)
39 Revolutions per minute (abbrev.)
41 The "a" in a.m.
42 Group of people working together
43 Male given name
44 Landing place, quay
46 Organ of vision
48 ISO country code for Bolivia
49 North West (abbrev.)
50 Circa (abbrev.)

Solution hint for prize competition of BioNews No. 49

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Send us the solution until **October 31, 2018**, via e-mail to **bionews@eppendorf.de**, or participate online at **www.eppendorf.com/bn-service**.

25 YEARS ANNIVERSARY

Science Congratulates Eppendorf BioNews on its 25th Anniversary

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