APPLICATION NOTE No. 447

# HEK293 Suspension Cell Culture Using the BioFlo® 320 Bioprocess Controller with BioBLU® 3c Single-Use Bioreactors

**Jorge L. Escobar Ivirico and Ma Sha**
Eppendorf, Inc., Enfield, CT, USA

Contact: bioprocess-experts@eppendorf.com

## Abstract

Mammalian cells cultivated *in vitro* represent one of the most important manufacturing platforms for vaccine and gene therapy developers. Especially, human embryonic kidney 293 (HEK293) cells are an attractive and reliable host for numerous biotherapeutic platforms. HEK293 cells have a wide variety of advantages including low-maintenance, rapid proliferation, and convenient application to both, transient and stable expression. Furthermore, they are easy to transfect and can produce large amounts of recombinant proteins and virus particles.
However, a major limitation of the cell line is its tendency to clump when converted to suspension format and therefore has been limited to adherent cell culture. To achieve large scale protein production, a new suspension-adapted HEK293 cell line, Expi293F™, was developed by Thermo Fisher. The new HEK293 cell line appears to be a robust suspension cell line capable of achieving greater per cell productivity in high density culture without clumping.
In this study, we evaluated the cell line using bioreactor batch culture. We used a BioFlo® 320 bioprocess control system and a BioBLU® 3c Single-Use Bioreactor to carry out Expi293F batch culture in 2 L scale. In addition we monitored and analyzed the metabolites as well as cell density and viability during 11 days of culture.

## Introduction

Developing innovative preventive solutions through new vaccine technologies or gene therapy platforms that respond to new diseases is an important challenge in present day biotechnology. The gene therapy market is projected to reach more than $ 3 billion by 2023 and the global vaccine market is expected to reach $46 billion by 2022, pushing them to the apex of the biotechnology food chain [1-3]. To produce high-quality biotherapeutics, process development involves several demanding components including cost, cell line development, small scale exploration, effective scaling, and optimization of upstream/downstream processes.

The selection of the host cell is a key factor based on its capabilities and properties, including its ability to grow in suspension or adhere to a substrate. HEK293 is one of the most versatile mammalian cell lines with a wide range of applications including expression of recombinant proteins, antibodies and viruses. The HEK293 cell line was immortalized in 1973 by the integration of a ~4.3 kbp adenoviral 5 (Ad5) genome fragment containing the E1A and E1B genes, located on chromosome 19 [4,5]. E1A and E1B are essential helper factors for adeno-associated virus (AAV) manufacture, making these cells a popular host platform for AAV particles production [6]. Our goal in this project was to evaluate the suspension culture of Expi293F cell line using Eppendorf bioprocess equipment and assess its suitability in virus production.

### HEK293 in gene therapy

Gene therapy involves the transfer of functional genes into cells to replace absent genes or correct defective ones. In a typical protocol, the cells are extracted from the donor and genetically modified by introducing a new or modified gene to inactivate or to replace a disease-causing gene. These modified cells are then reimplanted in the subject (ex vivo strategy). In addition, a well-established cell culture platform, such as HEK293, can be used to produce viral or non-viral delivery vehicles to introduce the gene of interest (GOI).

A well-established method (transient transfection) is frequently employed to produce different vectors using adherent human HEK293 cells cultivated in T-flasks or bioreactors. Prominent examples include lentivirus [7, 8], adenovirus [9,10], non-viral vectors [11], and AAV [12,13]. AAV represents one of the leading platforms for gene therapy due to its ability to provide in vivo long-term gene expression. Nearly 200 AAV clinical trials and biotherapeutic protocols are in different stages of FDA review, in which the transient transfection of adherent HEK293 cells has been the predominant platform [14,15]. We believe that the Expi293F cell line is a significant improvement over traditional HEK293 cell lines due to its robust growth under suspension culture conditions as well as its property of stable expression in addition to transient expression.

### HEK293 in vaccine production

During the last century, vaccines saved billions of lives throughout the world. Vaccine technology has distinguished itself as the most important development in the history of medicine. This unparalleled success has driven researchers to explore new and more efficient platforms to meet the constantly expanding demands of the industry.

Conventional vaccines usually contain whole weakened or inactivated viruses or protein subunits made by the pathogen to trigger an immune response. The cell-based vaccine platform is a well-established technology [16], offering several notable advantages:

> cell lines are well characterized and may be easily stored for future applications,
> their use avoids dependence on embryonated chicken eggs (ECE), whose quality is highly and unpredictably variable, despite their being the most common method used to develop vaccines,
> some viruses grow better in cells reducing the time to achieve high growth profiles [17],
> compared to ECE, viruses propagated in mammalian cells have shown an antigenic profile similar or identical to that of the field virus [18], and
> scalability is superior to the ECE production platform [19].

Although numerous mammalian cell lines have been evaluated for vaccine production [20-24], and performance in gene therapy protocols, the HEK293 cell line is one of the most widely used cell platforms for these demands. Specifically, high yield adenoviral vectors (~4 x $10^{15}$ viral particles) have been obtained in stirred-tank bioreactor systems using microcarriers [25,26]. Adherent HEK293 cells are easy to cultivate at laboratory scale, and require less expert bioengineering know-how, but when biotherapeutics production increases, suspension cell lines offer advantages in terms of scalability and robustness, using established stirred-tank bioreactor platforms. Eppendorf bioprocess systems, including BioFlo 320, are not medical devices and cannot be directly used for Gene Therapy and Vaccine production without special approval process. However, it can be used for effective HEK293 suspension cell culture and virus production research including research in the Gene Therapy and Vaccine production field at R&D level. To this end,

Expi293F cells (a suspension adapted HEK293 cell line) can accelerate the therapeutics development by enabling rapid, high yield and scalable production of proteins, viral antigens and AAV particles.

In this study, we used a BioBLU 3c Single-Use Bioreactor for Expi293F cell expansion and a BioFlo 320 as the bioprocess control system (Figure 1). We analyzed the cell growth, the viability as well as the metabolic activity (levels of glucose, ammonia and lactate in the medium).


**Fig. 1:** BioBLU 3c Single-Use Bioreactor (left) and BioFlo 320 bioprocess control system (right).

### Technical Features

In this study, pH, DO, and temperature were controlled online. Get to know more about the possibilities for bioprocess control with the BioFlo 320.

www.eppendorf.group/bioflo320

## Material and Methods

### Cell line and medium

We cultured the suspension Expi293F cell line (Thermo Fisher Scientific, USA) in Expi293 Expression Medium (Thermo Fisher Scientific, USA) formulated with GlutaMAX-I reagent. Expi293 Expression Medium is a chemically defined, serum and protein-free medium, ready to use without need for additional supplements.

### Inoculum preparation

We rapidly thawed the cryovial containing 1 mL of Expi293F cells (Thermo Fisher Scientific, A14527) at 1 x $10^7$ cells/mL, from a previously prepared cell bank, using a ThawSTAR® CFT2 instrument (MedCision®, USA). Just before the cells were completely thawed we decontaminated the vial wiping it with 70% alcohol before opening it in a laminar flow hood. We transferred the entire content of the cryovial into a 125 mL disposable, sterile and vented shaker flask containing 30 mL (24 % of the total volume) of pre-warmed Expi293 Expression Medium (Thermo Fisher Scientific, A1435101). We cultured the cells in a New Brunswick™ S41i $CO_2$ incubator Shaker (Eppendorf, Germany) at 37 °C, 8 % $CO_2$ and at agitation speed of 125 rpm. We cultured the cells for 4 days after thawing and then determined the cell viability and total viable cells using a Vi-CELL XR cell viability analyzer (Beckman Coulter). We then performed the subsequent passages when the viable cell density reached around 3 x $10^6$ cells/mL (typically 4 days after shaker flask inoculation) reaching more than 900 x $10^6$ cells in the third passage. During the expansion process we kept the inoculation density, percentage fill of the shake flasks and other parameters constant. Finally, we prepared the inoculum containing 800 x $10^6$ cells in 200 mL of Expi293F Expression Medium. The cell expansion workflow is shown in Figure 2.

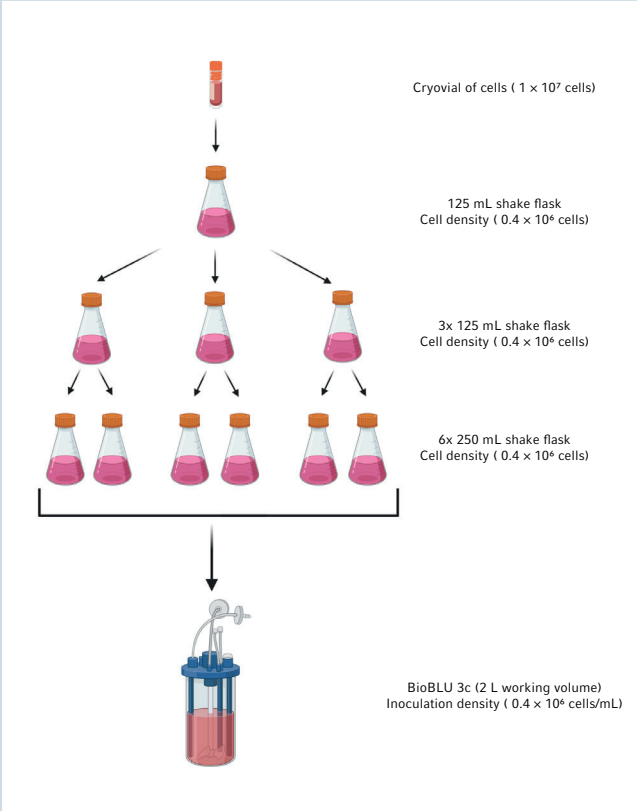


**Fig. 2: Inoculum preparation.**

**Created with: BioRender.com.**

### Bioreactor control

We used a BioFlo 320 bioprocess control station to perform two batch cultures using BioBLU 3c Single-Use Bioreactors equipped with a single pitched-blade impeller. The bioreactor unit is equipped with two universal port connectors for pH (port 1) and DO (port 2) sensors, an heat blanket connection providing precise temperature control, agitation control and a gas module that includes 3 TMFC, high-flow sparge drawer with a gas flow range of 0.04 – 20 SLPM.

### Sensor calibration

Prior to the preparation of the BioBLU 3c Single-Use Bioreactors, we connected the gel-filled pH sensor to the BioFlo 320 bioprocess controller. The software automatically detects the connected sensors to support an efficient workflow. We performed the calibration process according to the operation's manual using buffer solutions of pH 7 and pH 4 as "zero" and "span" respectively. Then, we disconnected the pH sensor and sterilized it in an autoclavable pouch.

### BioBLU 3c Single-Use Bioreactor preparation and process parameters

We equipped the BioBLU 3c with a magnetic drive, the previously sterilized pH sensor, inserted in a spare PG 13.5 port under aseptic conditions in the Biosafety Cabinet, a polarographic DO sensor (Mettler Toledo®), an exhaust condenser, a 3-gas mixing line connected to the gas sparge port, and 3 liquid addition ports (one for inoculation/glucose addition, one for base addition and another for the addition of 0.1 % of antifoam (Pluronic®-F68 surfactant, Life Technologies®, 24040-032). Then, we controlled the temperature using a heat blanket. Finally, we introduced the 1.8 L of Expi293F Expression Medium into each bioreactor and conditioned for at least 24 hours under the parameters and setpoints listed in Table 1.

**Table 1: Process parameters and setpoints of the first and second experiments.**

| Parameters | Setpoints |
|---|---|
| Starting volume | 1.8 L |
| Ending volume | 2 L |
| Initial agitation | 120 rpm (0.4 m/s tip speed) |
| Temperature | 37 °C |
| Inoculation density | 0.4 x $10^6$ cell/mL |
| Cell culture medium | Expi293™ Expression Medium |
| DO Setpoint | 40% (P=0.1; I=0.001) |
| pH Setpoint | 7.0 (deadband = 0.2), cascade to $CO_2$ (acid) and cascade to 0.45 M sodium bicarbonate (base) |
| Gassing range | Air flow: 0.04 SLPM -1 SLPM<br>$O_2$ flow: 0 SLPM -1 SLPM |

### Expi293FTM cells culture on BioBLU 3c Single-Use Bioreactor

We inoculated the BioBLU 3c Single-Use Bioreactors with the inoculum described above (see section "Inoculum preparation") reaching 2 L as working volume with a cell density around 0.4 x $10^6$ cell/mL and more than 95 % of cell viability. We monitored the temperature at 37 °C and controlled the dissolved oxygen (DO) at 40 % using the 3-Gas Auto mode. In addition, we limited the oxygen flow to 0 – 1 SLPM and the air flow to 0.04 – 1 SLPM in the controller setup screen to avoid high gas flow that can cause DO fluctuation and excessive foaming in the beginning stage of the cells culture. In addition to the gas flow limit, we added Pluronic-F68 surfactant as needed. We used a gel-filled pH sensor to control the pH during the cell culture 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 bioreactor daily and measured the pH, the cell viability and density as well as the concentration of various metabolites offline.

### Cell viability and metabolic activity

We collected samples on a daily basis from the BioBLU 3c Single-Use Bioreactors to determine the cell viability, cellular density, and the concentration of metabolites (glucose, ammonia ($NH_3$) and lactate), by connecting a sterile 5 ml syringe to the Luer Lock sample port. Then, we discarded 5 mL of dead volume and collected again 3 mL (using a new 5 mL sterile syringe) as a viable sample for analysis. We used 1 mL to measure the metabolite levels employing a Cedex® Bio Analyzer (Roche, USA), 1 mL to measure the cell viability and density using a Vi-Cell® XR Viability Analyzer (Beckman Coulter®, USA) and 1 mL to check the pH offline using an Orion Star A211 pH meter (Thermo Fisher Scientific, USA), which we calibrate daily using standard pH buffers.

## Results and Discussion

To evaluate the Expi293F suspension culture robustness, we performed two bioreactor batch culture runs using BioBLU 3c Single-Use Bioreactors controlled by BioFlo 320 bioprocess controller. We used Expi293 Expression Medium with additional glucose supplementation to extend the growth phase and increase the peak cell density. The inoculum was ready after the cell expansion in the New Brunswick S41i $CO_2$ incubator Shaker at 37 °C and 8 % $CO_2$ and agitation speed of 125 rpm. We then inoculated the BioBLU 3c Single-Use Bioreactor with an initial cell density of 0.4 x $10^6$ cells/mL under a controlled environment (see Table 1).

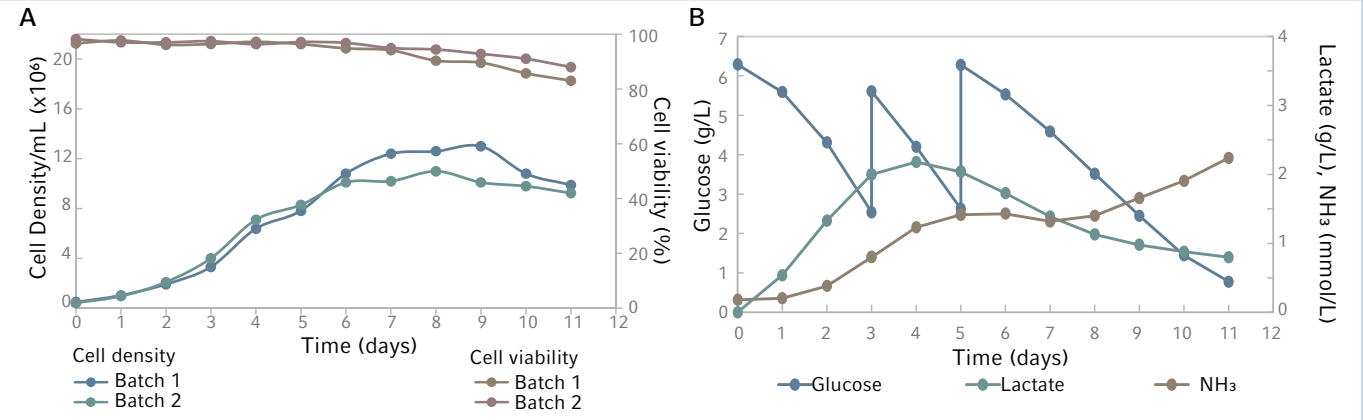


**Fig. 3** : Expi293F growth profile in BioBLU 3c Single-Use Bioreactor in Expi293 Expression Medium.
A: Expi293F cell density and viability. B: Metabolic profile.

In addition, we added Pluronic-F68 surfactant (0.1 %) to the medium in the bioreactor to decrease foaming produced by the gas introduced through the sparger.

As shown in Figure 3A, we observed a rapid increase of cell growth between days 1 and 9 of culture, reaching a peak in viable cells density at 13 x $10^6$ cells/mL, followed by a decrease in cell density and viability as anticipated. Furthermore, we determined the consumption of glucose and production of lactate and $NH_3$ while at the same time maintaining the concentration of lactate and $NH_3$ below 2 g/L and 2 mmol/L respectively during the whole run (See Figure 3B). We performed bolus glucose supplementation (to maintain target concentration > 2 g/L in both runs) at days 3 and 5 to extend the growth phase. The ammonia concentration gradually increased every day up to 2.2 mmol/L on day 11. We believe the depletion of glucose and other nutrients contributed to the decrease of the cell density, starting from day 9. Overall, cell growth increased around 32-fold.

## Conclusions

Using the BioFlo 320 bioprocess control system and BioBLU 3c Single-Use Bioreactors, we demonstrated the feasibility of applying glucose-enhanced batch culture technique to expand Expi293F cells rapidly up to 13 million cells/mL within 9 days. The efficient and straightforward configuration of the BioFlo 320 allows precise control of the cell culture environment, leading to reliable cell expansion. Although the experiments were conducted as feasibility studies and no optimization of conditions was attempted, we observed vigorous growth of Expi293F in suspension culture at a pace and simplicity close to Chinese hamster ovary (CHO) cells, the accepted industry standard. We believe that the Expi293F cell line has great potential in both vaccine and gene therapy method development when used in conjunction with Eppendorf's advanced stirred-tank bioreactors.

## Literature

[1] Grand View Research, "Gene Therapy Market Size, Share & Trends Analysis," 2019.
[2] Markets and Markets, "Gene Therapy Market by Vectors," 2019.
[3] Vaccines Market by Indication, Type, Route of Administration & Geography / Global Vaccines Market Forecasts 2018-2025 / ResearchandMarkets.com/CoherentMarketInsights, 2018.
[4] Graham FL, Smileyt J, Russell WC, Nairn R. Characteristics of a Human Cell Line Transformed by DNA from Human Adenovirus Type 5. J. gen. Virol 1977; 36:59–7.
[5] Louis N, Evelegh C, Graham FL, 1997. Cloning and Sequencing of the Cellular– Viral Junctions from the Human Adenovirus Type 5 Transformed 293 Cell Line. Virology 1997; 233:423–429.
[6] Clément N, Grieger JC. Manufacturing of recombinant adeno-associated viral vectors for clinical trials. Mol. Ther. Methods Clin. Dev. 2016; 3:16002.
[7] McCarron A, Donnelley M, McIntyre C, Parsons D. Transient Lentiviral Vector Production in HEK 293T Cells Using the BioFlo® 320 Control Station with a BioBLU® 5p Single-Use Packed-Bed Vessel. Application Note 2019; App-411.
[8] Ferreira CB, Sumner RP, Rodriguez-Plata MT, Rasaiyaah J, Milne RS, Thrasher AJ, Qasim W, and Towers GJ. Lentiviral Vector Production Titer Is Not Limited in HEK293T by Induced Intracellular Innate Immunity. Molecular Therapy: Methods & Clinical Development. 2020; 17:209-219.
[9] Feng L, Wang Q, Shan C, Yang C, et al. An adenovirus-vectored COVID-19 vaccine confers protection from SARS-COV-2 challenge in rhesus macaques. Nat Commun. 2020; 11:4207.
[10] Fedosyuk S, Merritt T, Peralta-Alvarez MP, Morris SJ, et al. Simian adenovirus vector production for early-phase clinical trials: A simple method applicable to multiple serotypes and using entirely disposable product-contact components. Vaccine. 2019; 37:6951–6961.
[11] Patil S, Gao YG, Lin X, et al. The Development of Functional Non-Viral Vectors for Gene Delivery. Int J Mol Sci. 2019; 20:5491.
[12] Wang D, Tai PWL, Gao G. Adeno-associated virus vector as a platform for gene therapy delivery. Nat Rev Drug Discov. 2019; 18:358–378.
[13] Li C, Samulski RJ. Engineering adeno-associated virus vectors for gene therapy. Nat Rev Genet. 2020; 21:255–272.
[14] Rumachik NG, Malaker SA, Poweleit N, et. Methods Matter: Standard Production Platforms for Recombinant AAV Produce Chemically and Functionally Distinct Vectors. Molecular Therapy: Methods & Clinical Development. 2020; 18:98-118.
[15] Grimm D, Kern A, Rittner K, Kleinschmidt JA. Novel tools for production and purification of recombinant adeno-associated virus vectors. Hum. Gene Ther.1998; 9:2745–2760.
[16] Le Ru A, Jacob D, Transfiguracion J, Ansorge S, Henry O, Kamen AA. Scalable production of influenza virus in HEK-293 cells for efficient vaccine manufacturing. Vaccine. 2010; 28:3661–3671.
[17] Jung EJ, Lee KH, Seong BL. Reverse genetic platform for inactivated and live-attenuated influenza vaccine. Exp Mol Med. 2010; 42:116-21.
[18] Donis RO. The Influenza Cell Culture Working Group. Performance characteristics of qualified cell lines for isolation and propagation of influenza viruses for vaccine manufacturing. Vaccine. 2014; 32:6583-90.
[19] Hegde NR. Cell culture-based influenza vaccines: A necessary and indispensable investment for the future. Hum Vaccin Immunother. 2015; 11:1223–1234.
[20] Rimmelzwaan GF, Baars M, Claas ECJ, Osterhaus ADME. Comparison of RNA hybridization, hemagglutination assay, titration of infectious virus and immunofluorescence as methods for monitoring influenza virus replication in vitro. Journal of Virological Methods. 1998; 74:57–66.
[21] Voeten JTM, Brands R, Palache AM, et al. Characterization of high-growth reassortant influenza A viruses generated in MDCK cells cultured in serum-free medium. Vaccine. 1999; 17:1942–1950.
[22] Pau MG, Ophorst C, Koldijk MH, Schouten G, Mehtali M, Uytdehaag F. The human cell line PER.C6 provides a new manufacturing system for the production of influenza vaccines. Vaccine. 2001; 19:2716–2721.
[23] Kistner O, Barrett PN, Mundt W, Reiter M, Schober-Bendixen S, Dorner F. Development of a mammalian cell (Vero) derived candidate influenza virus vaccine. Vaccine. 1998; 16:960–968.
[24] Youil R, Su Q, Toner TJ, et al. Comparative study of influenza virus replication in Vero and MDCK cell lines. Journal of Virological Methods. 2004; 120:23–31.
[25] Vemula SV, Mittal SK. Production of adenovirus vectors and their use as a delivery system for influenza vaccines. Expert Opinion on Biological Therapy. 2010; 10:1469–1487.
[26] Fuenmayor Garcés J, Gutiérrez Granados S, Gòdia Casablancas F, Cervera Gracia L. Cell line and plasmid influence on the efficiency of HEK293 cells transient transfection for the production of VLP-based vaccines. New Biotechnology. 2016; 33:S32-S33.

**Ordering information**

| Description | Order no. |
|---|---|
| **BioFlo® 320,** base control station, no water connection | 1379962911 |
| **BioFlo® 320,** left-handed orientation/four front-mounted peristaltic pumps (3 @ 5 – 25 rpm/1 @ 20 – 100 rpm) | 1379963211 |
| **BioFlo® 320,** sparge gas option, 3 TMFC (0.04 – 20 SLPM) | 1379501311 |
| **Accessories** | |
| **Touchscreen Monitor Bundle,** includes desk mount and 1 meter cable set, for BioFlo® 320 | M1379-9906 |
| **Bioreactor** | |
| **Single-Use Vessel Bundle,** for BioFlo® 320, for BioBLU® 3c/5c | M1379-0322 |
| **BioBLU® 3c,** 1.25 L - 3.75 L, Microsparger, 1x pitched-blade | 1386000100 |
| **BioBLU® 3c** ,1.25 L - 3.75 L, Macrosparger, 1x pitched-blade | 1386000300 |
| **BioBLU® 3c,** 1.25 L - 3.75 L, Microsparger, 2x pitched-blade | 1386120000 |
| **BioBLU® 3c,** 1.25 L - 3.75 L, Macrosparger, 2x pitched-blade | 1386121000 |

**Your local distributor: www.eppendorf.com/contact**
Eppendorf SE · Barkhausenweg 1 · 22339 · Hamburg · Germany
eppendorf@eppendorf.com · www.eppendorf.com

www.eppendorf.com/bioprocess