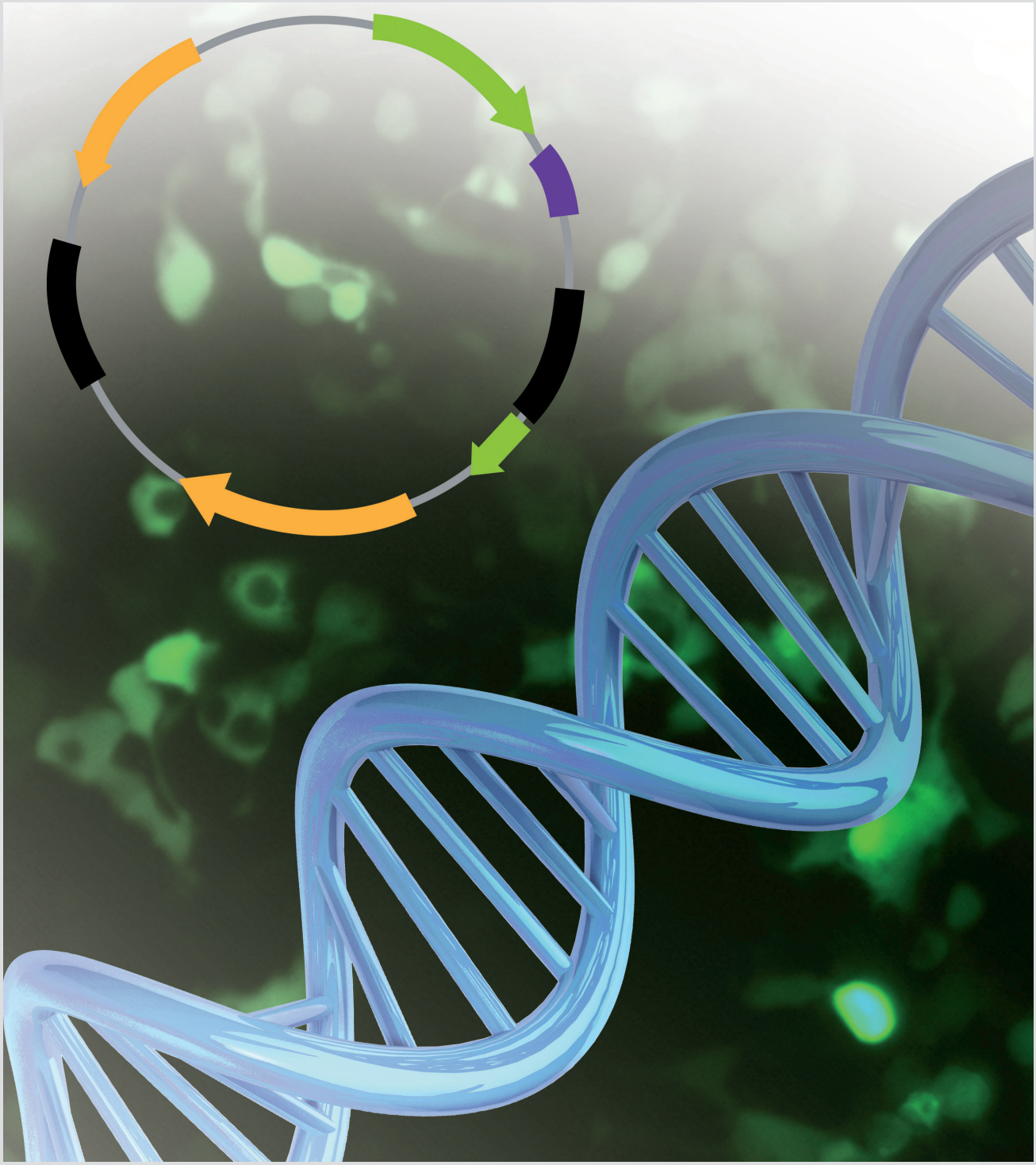
Application Note

Enhanced Transfection Efficiency on Protein Coated Microplates



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1 Introduction

Although viral vectors have been proven to be efficient means of delivering genetic material, there are several detriments associated with their use. Especially when utilized *in vivo*, viral vectors are known to cause adverse immune responses and toxic reactions[1].
In consequence, synthetic delivery systems have become an increasingly attractive alternative for viral vectors. However, there are still critical limitations with synthetic delivery systems, primarily low transfection efficiencies and cytotoxic effects.
To resolve these issues, several studies have been performed to determine adequate means of improving cell vitality following transfection as well as the efficiency of the transfection itself[2].
In this study, the effect of culturing cells on protein coated surfaces to enhance cell viability and improve overall transfection efficiency was examined.

Protein coated culture vessels are widely used in cell culture to increase adhesion of cells, differentiation, vitality and proliferation. Two types of proteins/polypeptides are used to coat polystyrene surfaces for this purpose.
Extracellular matrix proteins – like Collagen type I, Fibronectin and Laminin – mediate specific binding of the cell to the protein. Fibronectin and Laminin exhibit a binding sequence – the R-G-D (Arg-Gly-Asp) motif – known to bind to the integrin receptor of the cell. This heterodimeric cell surface adhesion receptor not only regulates binding between cell and substrate, it also triggers signaling pathways that direct cellular processes such as proliferation, survival, differentiation and migration[3].

Poly-D-Lysine, a synthetically produced biomolecule, belongs to the non-specific adhesion-promoting polypeptides. PDL is frequently used to promote cell adhesion, especially during washing steps, as well as to enhance cell vitality and proliferation during serum-reduced or serum-free cultivation.

In the present study, the impact of cell cultivation substrate on transfection efficiency was evaluated. The investigation included various cell types cultured on Poly-D-Lysine and Collagen type I coated surfaces along with a physically treated cell culture surface.
Two different reporter systems were used to assess transfection efficiency:
The green fluorescent protein (GFP) has gained widespread importance to visualize spatial and temporal patterns of gene expression both *in vitro* and *in vivo*. It can be evaluated qualitatively by fluorescence imaging as well as quantitatively by flow cytometry[4] to demonstrate transfection efficiency in an experimental approach.
Especially as fusion protein, the GFP tagged protein provides useful insights for expression and location of a specific protein in the cell.

Gaussia Luciferase is a reporter luciferase from the marine copepod Gaussia princeps. Using the reporter plasmid pCMV-Gluc Gaussia Luciferase can be expressed in mammalian cells.
This Luciferase catalyzes the oxidation of the substrate coelenterazine in a reaction that emits light at a wavelength of 470 nm[5]. Transfection efficiency can therefore be easily detected by measuring Luciferase actively in the culture supernatant via luminescence reading.

2 Materials

2.1 Reagents and disposables

Item	Manufacturer	Cat.-No.
Lipofectamine 2000 transfection reagent	Invitrogen	11668-027
Gaussia Luciferase Assay Kit	New England Biolabs	E3300L
pCMV-GLuc control plasmid	New England Biolabs	N8081S
pcDNA3-EGFP	Generously provided from the Institute of Cell Biology and Immunology, Stuttgart, Germany	
DMEM	Biochrom AG	F0435
RPMI	Biochrom AG	F1295
OptiMem-Medium	Invitrogen	31985
Fetal calf serum	Biochrom AG	S0415
Cell culture treated 96 well microplates	Greiner Bio-One GmbH	655 090
CELLCOAT® PDL-coated 96 well microplates	Greiner Bio-One GmbH	655 946
CELLCOAT® Collagen-coated 96 well microplates	Greiner Bio-One GmbH	655 956
96 well microplates, white	Greiner Bio-One GmbH	655 073
Pipette tips, 200 µl	Greiner Bio-One GmbH	685 261
Serological Pipettes, 10 ml	Greiner Bio-One GmbH	607 180

2.2 Equipment

Item	Manufacturer
Multipipette	Eppendorf
Luminescence Reader	Tecan, SpectraFluor Plus
Fluorescence Microscope	Leica
Tissue culture bench	Fisher Scientific GmbH, Hera Safe

2.3 Cell culture medium

Cell type	Medium content
CHO	RPMI + 10% FCS
HEK-293	DMEM + 10% FCS
HepG2	RPMI + 10% FCS + 2% Glutamine

3 Methods

3.1 Transfection

Cells were seeded on PDL-coated-, Collagen type I-coated and cell culture treated 96 well microplates in antibiotic free culture medium one day prior to transfection. To reach confluency the next day, the following cell numbers were used:

CHO	30,000 cells/well
HEK-293	75,000 cells/well
HepG2	75,000 cells/well

Cells were transfected with the pcDNA3-EGFP for green fluorescence protein (GFP) expression as well as with pCMV-GLuc for the luciferase assay in two different approaches.
0.32 µg plasmid DNA was diluted in 25 µl OptiMem-Medium (vial A). In parallel 1 µl of Lipofectamine was diluted in 24 µl OptiMem-Medium (vial B).
After 5 min incubation at room temperature, the content of vial B was carefully mixed with the diluted plasmid DNA in vial A.
After 20 min incubation the transfection reagent was pipetted onto the cells (50 µl/well). Medium was exchanged with antibiotic-free culture medium after 4 hours.

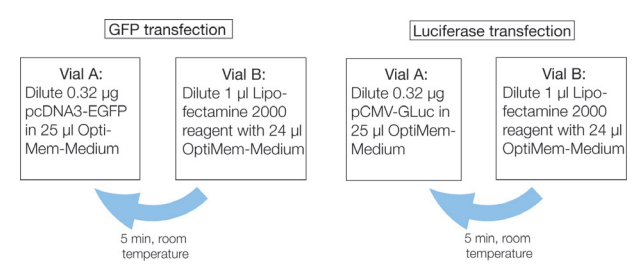

Figure 1a): Preparation of transfection reagent

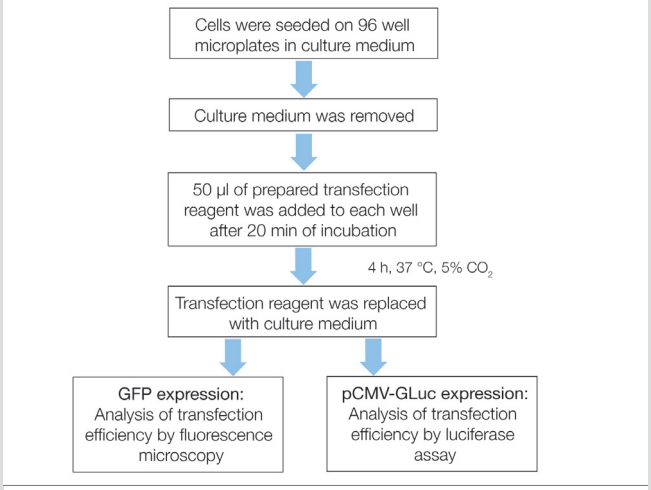

Figure 1b): Transfection protocol

3.2 GFP-expression

Cells were observed microscopically before transfection one day after seeding. For GFP expression, images were taken with a fluorescence microscope at day 2 after transfection.

3.3 Luciferase assay

20 µl of cell culture supernatant was transferred to a white 96 well microplate. For preparing the Luciferase reagent, 30 µl of Luciferase reagent was diluted in 3 ml buffer. 50 µl was pipetted with a multichannel pipette to each well containing cell culture supernatant. As signal intensity decreases over time, luminescence was measured immediately.

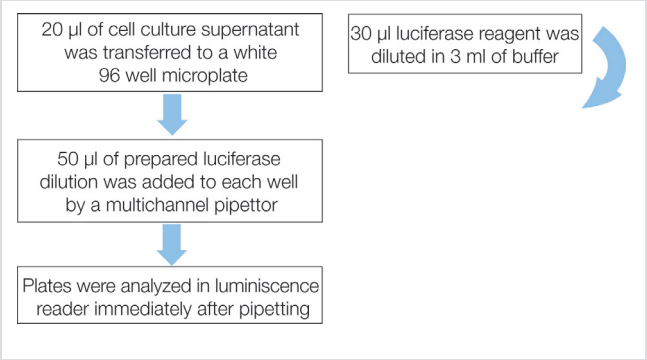

Figure 2: Luciferase assay protocol

4 Results

HEK-293 cells were seeded on Collagen type I and Poly-D-Lysine (PDL)-coated surfaces and transfected one day after seeding with the pcDNA3-EGFP and pCMV-GLuc plasmid in two different approaches. Transfection efficiency on these surfaces was compared to cell culture treated surface by fluorescence imaging and evaluation of luciferase activity, respectively.

4.1 Cell attachment of HEK-293 cells on protein coated plates before and after transfection

Cell morphology and number were assessed prior to and one day after transfection. As can be seen in the following images (Fig. 3), cell number as well as attachment properties decreased following transfection, corresponding with known related cytotoxic effects. The cytotoxic effects of transfection were reduced when cells were cultured on protein coated plates. Even if cell number slightly decreased, cells demonstrated a typical adherent morphology on PDL- (Fig. 3D) and on Collagen Type I-coated (Fig. 3F) plates when compared to standard cell culture treated microplates (Fig. 3B).

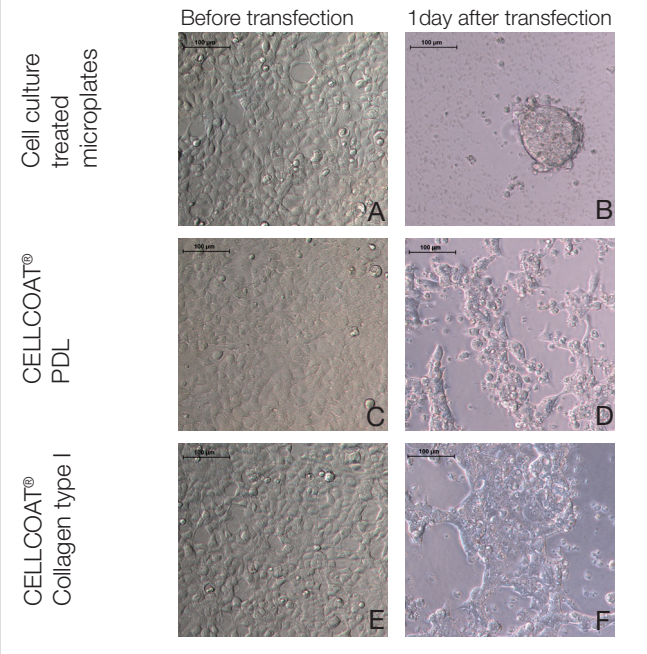

Figure 3: Cell attachment of HEK-293 cells on protein coated and standard cell culture treated microplates before and after transfection

4.2 GFP-transfection of HEK-293 cells 2 days after transfection

GFP expression was checked two days after transfection by fluorescence imaging. HEK-293 cells could be well transfected with this construct and showed high transfection efficiency on Collagen type I- (Fig. 4F) and PDL-coated (Fig. 4D) compared to standard cell culture treated microplates (Fig. 4B).

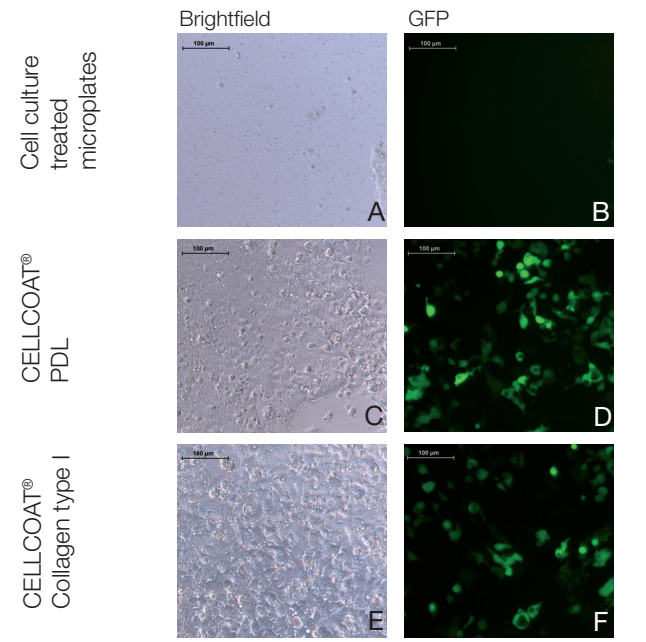

Figure 4: GFP transfection of HEK-293 cells on cell culture treated, PDL-coated and Collagen type I-coated surfaces. Brightfield and the corresponding fluorescence images showed the transfection efficiency of HEK-293 cells.

4.3 Luciferase activity of cells seeded on different surfaces after transfection

4.3.1 Transfection efficiency of HepG2 cells

Efficiency of transfection of pCMV-GLuc was determined based on luminescence activity of transfected HepG2, CHO and HEK-293 cells. HepG2 displayed the highest luminescence signal corresponding to transfection rate on the Greiner Bio-One PDL-coated surface (Fig. 5), indicating this as the most appropriate surface for transfecting these cells.
In general transfection efficiency improved over the entire experiment. This effect can be explained due to increased transfection efficiency as well as proliferation of transfected cells.

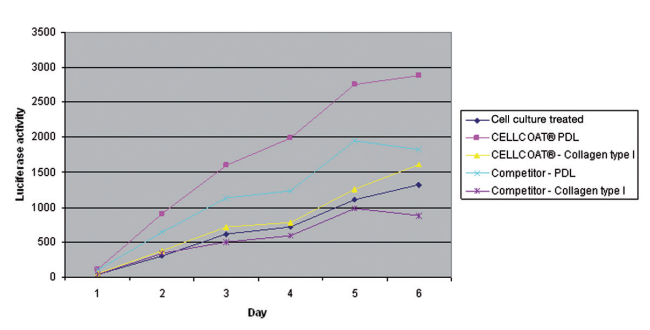

Figure 5: Time course of Luciferase activity of HepG2 cells on cell culture treated, PDL-coated and Collagen type I-coated microplates

4.3.2 Transfection efficiency of CHO cells

CHO cells also displayed a higher transfection rate with CELLCOAT® plates than with standard cell culture treated plates. The cells demonstrated the highest transfection efficiency when cultured on Collagen type I coated microplates, whereas PDL-coated microplates exhibited the same performance as cell culture treated plates in terms of transfection. Transfection efficiency reached a plateau four days after transfection.

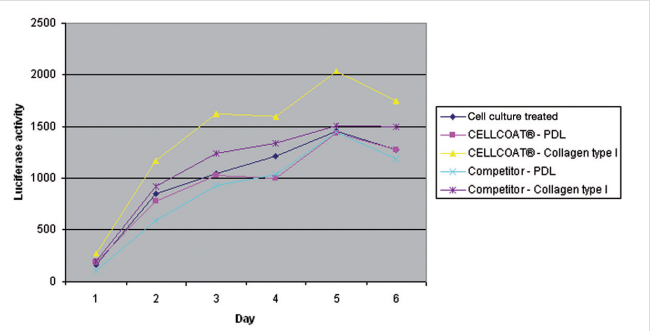

Figure 6: Time course of Luciferase activity of CHO cells on cell culture treated, PDL-coated and Collagen type I-coated microplates

4.3.3 Transfection efficiency of HEK-293 cells

HEK-293 seems to be the ideal cell line using pCMV-GLuc transfection with Lipofectamine 2000 reagent. The transfection efficiency was very high on both PDL- and Collagen type I-coated plates. On standard cell culture microplates the toxic effects of transfection became highly apparent, as most of the cells detached following transfection (Fig. 7). Because of this, the luminescence signal intensity for standard cell culture microplates was quite low throughout the Luciferase assay experiment when compared to signal intensity for both PDL- and Collagen type I-coated microplates. Luciferase expression reached a plateau at day three after transfection.

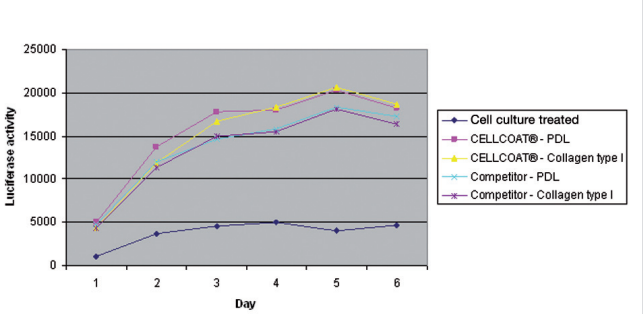

Figure 7: Time course of Luciferase activity of HEK-293 cells on cell culture treated, PDL-coated and Collagen type I-coated microplates

5 Summary & discussion

Transfection efficiency in HepG2, CHO and HEK-293 cells was evaluated using reporter plasmids pCMV-GLuc for Luciferase activity. HEK-293 cells were further transfected with pcDNA3-EGFP for GFP expression.
Cytotoxic effect during transfection process could be observed in different degrees on all surfaces. The number of cells clearly decreased on day one after transfection. However, adhesion of cells to PDL- and Collagen type I-coated substrates was significantly enhanced in comparison to standard cell culture treated surface. Cells cultured on CELLCOAT® surfaces demonstrated a typical expanded morphology, while HEK-293 cells detached after transfection on the standard cell culture surface.

Transfection of HEK-293 cells with the pcDNA3-EGFP was successfully accomplished on PDL- and Collagen type I-coated plates but not on cell culture treated microplates. This was primarily because cells on standard cell culture treated plates detached during transfection and were washed away during medium exchange. In contrast, cells cultured on CELLCOAT® protein coated microplates remained healthy post-transfection.

In general different cells could be well transfected when cultured on protein coatings. While HepG2 cells were best transfected on PDL-coated surface, Collagen type I coating was optimal for CHO cells. Both coatings – PDL and Collagen type I – performed well for HEK-293 cells.

The highest impact of cell culture substrate to transfection efficiency could be observed for HEK-293 cells. While nearly all cells detached following transfection on cell culture treated surface, transfection efficiency was high on both PDL- and Collagen type I-coated surfaces.
In summary, CELLCOAT® plates demonstrate excellent suitability for enhancing cell survival during the transfection process and improving overall transfection efficiency.

HepG2:		
CELLCOAT® PDL	>	CELLCOAT® Collagen/Cell culture treated
CHO:		
CELLCOAT® Collagen	>	CELLCOAT® PDL/Cell culture treated
HEK-293:		
CELLCOAT® PDL/Collagen	>	Cell culture treated

Figure 8: Ideal surfaces for transfection for HepG2, CHO and HEK-293 cells

6 Literature

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Revision: August 2009 - F073 103

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Germany (Main office)
Greiner Bio-One GmbH
Phone: (+49) 70 22 94 80
E-Mail: info@de.gbo.com

Austria
Greiner Bio-One GmbH
Phone: (+43) 75 83 67 91 0
E-Mail: office@at.gbo.com

Belgium
Greiner Bio-One BVBA/SPRL
Phone: (+32) 24 61 09 10
E-Mail: info@be.gbo.com

Netherlands
Greiner Bio-One B. V.
Phone: (+31) 1 72 42 09 00
E-Mail: info@nl.gbo.com

France
Greiner Bio-One SAS
Phone: (+33) 1 69 86 25 50
E-Mail: infos@fr.gbo.com

UK
Greiner Bio-One Ltd.
Phone: (+44) 14 53 82 52 55
E-Mail: info@uk.gbo.com

Japan
Greiner Bio-One Co. Ltd.
Phone: (+81) 3 35 05 88 75
E-Mail: info@jp.gbo.com

USA
Greiner Bio-One North America Inc.
Phone: (+1) 704-261-78 00
E-Mail: info@us.gbo.com

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