

# Optimization of TransFectin™ Lipid Reagent-Mediated Transfection for Different Cell Types

Angelika Bondzio, Nicole Walk, Jennifer Schön, and Ralf Einspanier  
Institute of Veterinary Biochemistry, Free University of Berlin, Berlin, Germany

## Introduction

The ability to introduce DNA into cells provides a powerful tool for studying the *in vivo* function and control of mammalian genes. Methods and reagents designed for nucleic acid delivery have received considerable attention, especially in protocols and discussions related to effective gene therapy strategies.

TransFectin lipid reagent is a mixture of a proprietary cationic lipid and colipid DOPE (1,2-dioleoylphosphatidylethanolamine). Cationic lipids, brought in contact with aqueous solutions under special conditions, form positively charged micelles or liposomes. These micelles associate with the negatively charged phosphates of nucleic acids and form spontaneous complexes with DNA or RNA. The lipid-DNA (or -RNA) complexes fuse or associate with the cell membrane via hydrophobic and electrostatic interactions, and the complex is then internalized (Zhou and Huang 1994, Remy et al. 1994).

It is important to optimize transfection conditions not only for each transfection method but also for every cell type. Cell density, duration of transfection, volume of medium during transfection, and ratio of lipid reagent to DNA are key factors for efficiency. In this article we describe the optimization of transfection with TransFectin monitored by a secreted alkaline phosphatase (SEAP) reporter gene assay. We compare two suspension cell types that are considered difficult to transfect: bovine peripheral blood mononuclear cells (PBMCs) and bovine B-lymphosarcoma BL3.1 cells (Anderson et al. 2004, Fenton et al. 1998). Additionally, we optimize the transfection efficiency for two adherent cell types: primary bovine oviductal cells and a calf pulmonary artery endothelial cell line (CPAE).

## Methods

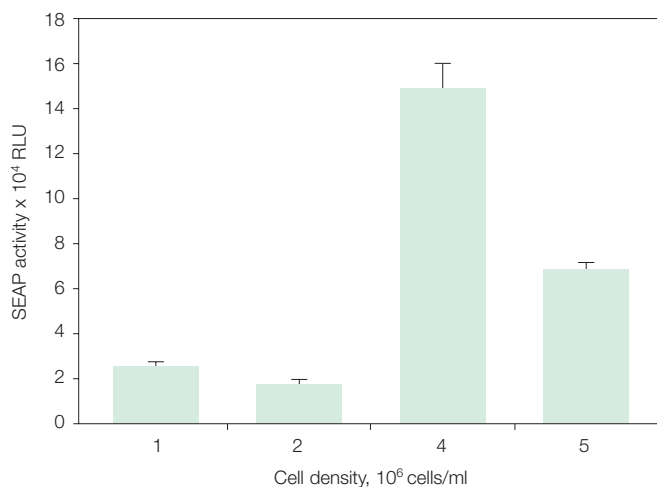
### Cell Cultures

Lymphocytes were prepared from heparinized bovine peripheral blood after 1:1 dilution with PBS and Ficoll-Paque PLUS (Amersham Biosciences) density gradient centrifugation. Cells were cultured in RPMI-1640 medium (supplemented with 25 mM glucose, 10 mM HEPES, 1 mM sodium pyruvate, and 10% FCS). The cell density was adjusted to  $4 \times 10^6$  cells/ml. PBMCs were transfected after 20–24 hr of cultivation. The B-lymphosarcoma cell line, BL3.1, was obtained from ATCC (CRL-2306). Cell density of suspension cells BL3.1 was adjusted to  $5 \times 10^5$ /ml 1 day prior to transfection in RPMI-1640 medium.

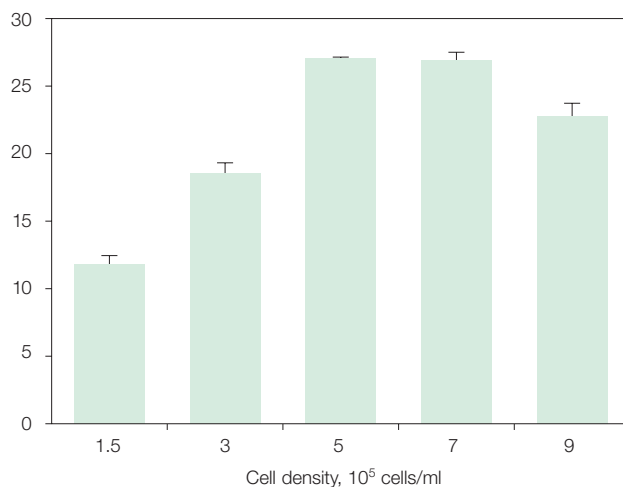
Bovine oviductal cells were freshly prepared as described previously (Ulbrich et al. 2003) with some modifications: After centrifugation ( $550 \times g$  for 5 min), 50  $\mu$ l cell pellet was diluted in 20 ml Medium 199 (supplemented with 20 mM HEPES, 0.68 mM sodium pyruvate, 2.3 mM L-glutamine, and 10% FCS). This preparation was designated the initial dilution, and further dilutions were prepared for optimization of cell density. The cultivated primary cells were transfected 1 day after they became adherent (4 days after preparation). The CPAE cells (ATCC, CCL-209) were plated in a concentration of  $5 \times 10^4$  cells/ml in MEM-Earle (supplemented with 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 2 mM glutamine, and 20% FCS) 1 day before transfection.

Each cell type was seeded on 24-well culture plates in 1 ml of the appropriate growth medium per ml and incubated at 37°C in a humidified 4.5% CO<sub>2</sub> atmosphere. The cell viability and proliferation were surveyed by colorimetric assay using WST-1 cell proliferation reagent (Roche) as recommended by the manufacturer.

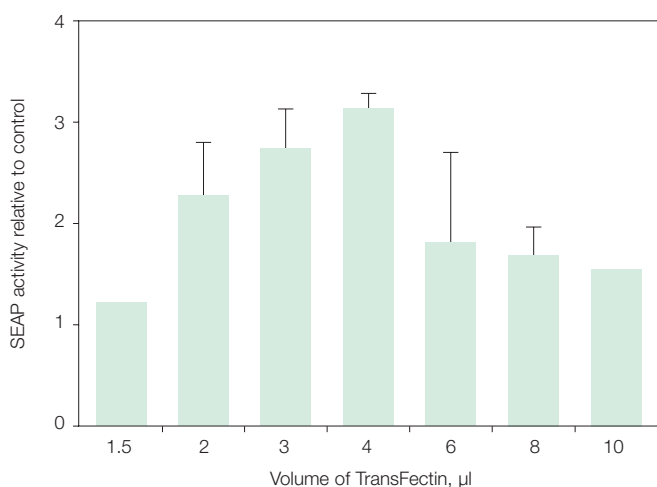
### A. PBMCs



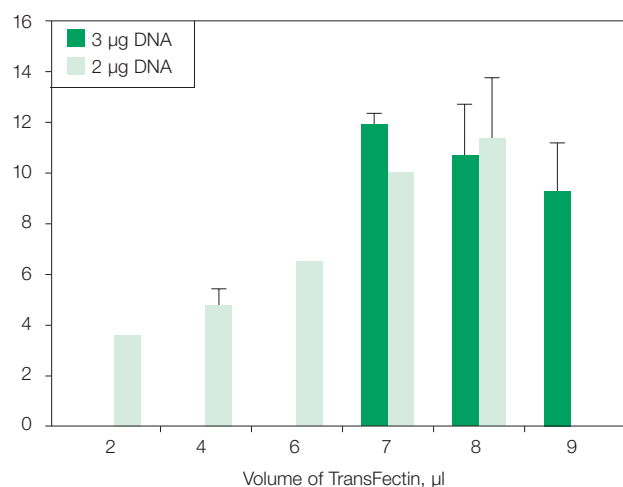
### C. BL3.1 cells



### B. PBMCs



### D. BL3.1 cells



**Fig. 1. Optimization of transfection conditions for suspension cells.** Conditions were optimized for PBMCs (A, B) and BL3.1 cells (C, D). To evaluate the effect of cell density on transfection efficiency, cells were seeded at indicated concentrations 24 hr prior to transfection using 2 µg DNA (pTAL-SEAP2-control) and 4 µl (A) or 7 µl (C) TransFectin. To evaluate the effect of the amount of TransFectin on transfection efficiency, PBMCs ( $4 \times 10^6$ /ml) were transfected using 2 µg DNA (pTAL-SEAP2-control) and indicated amounts of TransFectin (B). BL3.1 cells ( $5 \times 10^5$ /ml) were transfected using 2 or 3 µg DNA (D). Cell culture supernatant was assayed 24 hr after transfection for SEAP activity. Values are expressed relative to those for transfection with a promoterless control plasmid (error bars indicate standard deviation; A, n = 4; B, n = 6; C, n = 4; D, n = 7).

### Plasmids

Cells were transfected with the reporter plasmid TAL-SEAP2-control (Clontech), which contains the SEAP gene under control of the SV40 early promoter/enhancer or with a promoterless plasmid as negative control. In some cases cells were additionally transfected with a vector containing a  $\beta$ -galactosidase reporter gene under the control of the CMV promoter (CMV $\beta$ -Gal, kindly provided by Grant R MacGregor, Stanford University, USA).

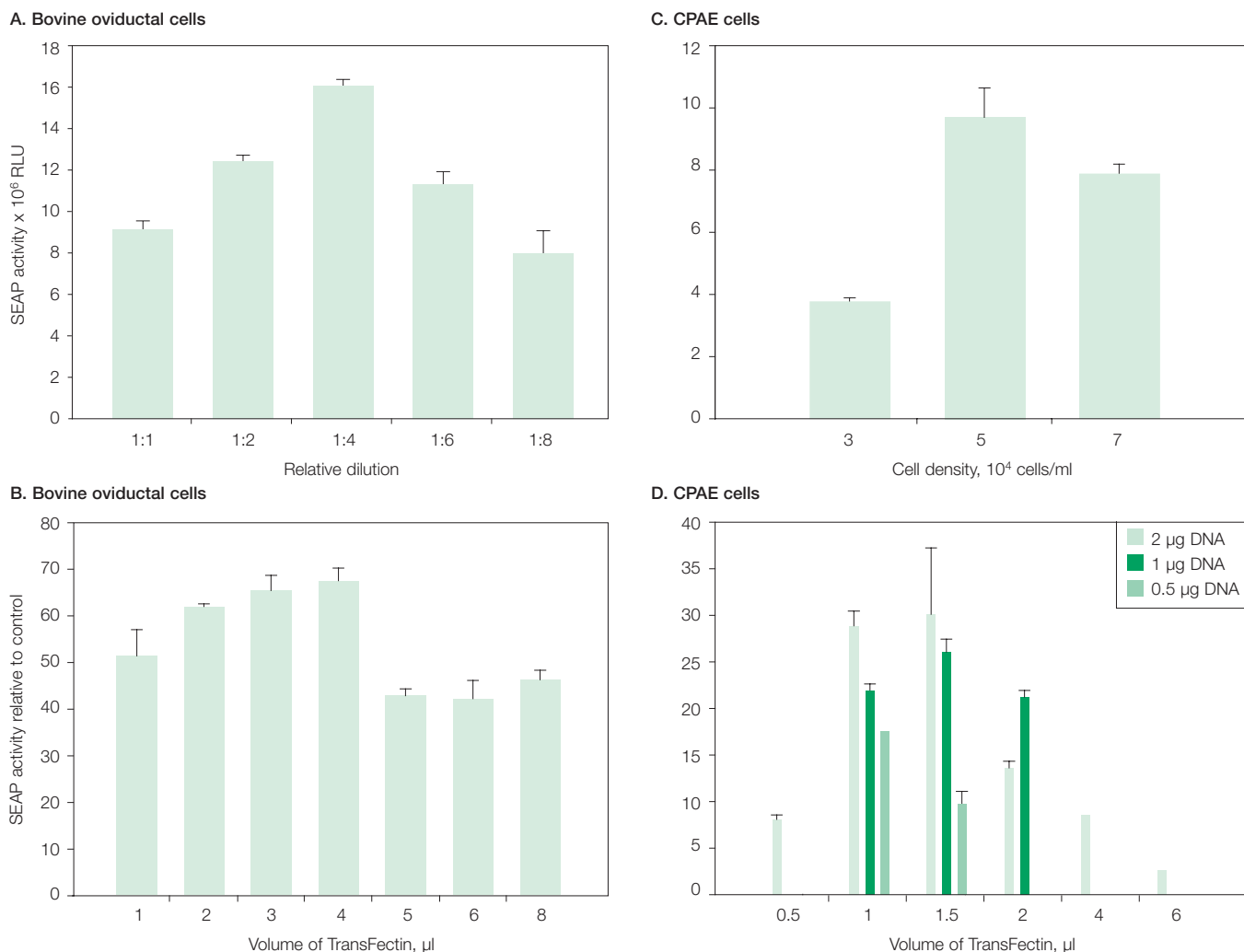
### Transfection Protocol for 24-Well Plate Format

For each well, DNA was diluted in 50 µl of serum-free growth medium and mixed gently briefly. TransFectin was prepared in 50 µl of serum-free growth medium per ml. Both solutions were combined, mixed gently, and incubated at room temperature for 20 min to allow TransFectin-DNA complex

formation. The TransFectin-DNA mixture (100 µl) was added to the growth medium in each well and mixed gently by rocking the plate. Cells were incubated at 37°C and 4.5% CO<sub>2</sub>.

### Detection of Transfection Efficiency

The relative transfection efficiency was assessed by measuring SEAP activity in the culture media using a chemiluminescent SEAP reporter gene assay (Roche) according to the manufacturer's protocol. For quantitation, 25 µl of the transfected cell culture supernatant was used. The resulting light signal was measured in a Sirius luminometer (Berthold). Values were expressed in relative light units (RLU) or as relative to the value from cells transfected with a promoterless plasmid. The  $\beta$ -galactosidase activity was estimated using a histochemical kit (Roche). Cells were stained with X-Gal 2 days after transfection.



**Fig. 2. Optimization of transfection conditions for adherent cells.** Conditions were optimized for primary bovine oviductal cells (A, B) and CPAE cells (C, D). To evaluate the effect of cell density on transfection efficiency, primary bovine oviductal cells (A) were seeded at different dilutions of the initial cell suspension (a 1:4 dilution of the initial cell suspension prior to seeding corresponds to a cell density of 3–5  $\times 10^4$  cells/ml in a 4 days' culture) and transfected using 2  $\mu$ g DNA (pTAL-SEAP2-control) and 4  $\mu$ l TransFectin. CPAE cells (C) were seeded at the indicated concentrations 24 hr prior to transfection using 2  $\mu$ g DNA and 2  $\mu$ l TransFectin. To evaluate the effect of the amount of TransFectin on transfection efficiency, primary bovine oviductal cells (3–5  $\times 10^4$ /ml) were transfected using 2  $\mu$ g DNA (pTAL-SEAP2-control) and indicated amounts of TransFectin (B). CPAE cells (5  $\times 10^4$ /ml) were transfected using 0.5, 1, or 2  $\mu$ g DNA (D). Cell culture supernatant was assayed 24 hr after transfection for SEAP activity. Values are expressed relative to those for transfection with a promoterless control plasmid (error bars indicate standard deviation; A, n = 4; B, n = 9; C, n = 4; D, n = 5).

## Results

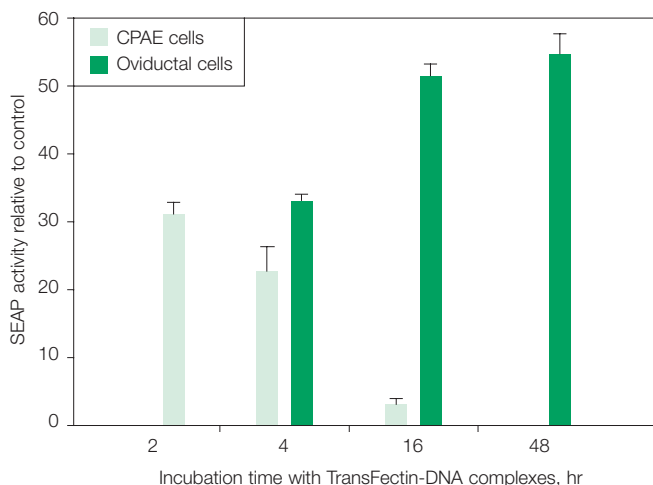
### Suspension Cultures

Transfection conditions were optimized for lymphoid cells (PBMC and BL3.1). TransFectin/DNA ratio as well as cell density are known to affect transfection efficiency. Therefore, we examined in initial experiments the impact of cell density for each cell type, and then optimized the TransFectin/DNA ratio. With PBMCs, the highest SEAP activity was achieved by seeding 4  $\times 10^6$  cells/ml 24 hr prior to transfection (Figure 1A). Figure 1B shows the results of TransFectin/DNA ratio optimization for PBMCs. The highest efficiency was achieved with 2  $\mu$ g DNA and 4  $\mu$ l TransFectin. Neither 1  $\mu$ g nor 3  $\mu$ g DNA resulted in higher efficiency on PBMCs at all tested TransFectin/DNA ratios (data not shown).

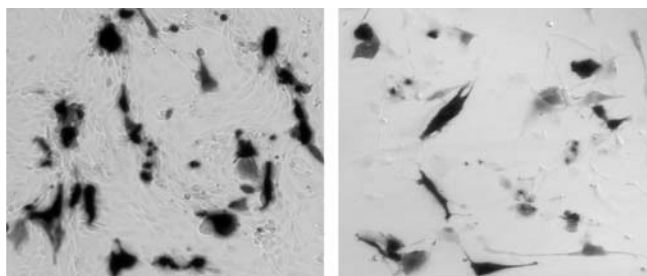
Similar experiments were carried out with BL3.1 cells in suspension. For these cells, the best results were obtained with 5–7  $\times 10^5$  cells/ml (Figure 1C). In comparison to the PBMCs, the established B-lymphosarcoma cell line, BL3.1, showed higher transfection efficiencies. The best results were achieved using 2–3  $\mu$ g DNA and 7–8  $\mu$ l TransFectin (Figure 1D).

### Adherent Cells

Primary bovine oviductal cells revealed highest transfection efficiencies at a cell density of 3–5  $\times 10^4$  adherent cells per ml (4 days' culture). This corresponded to a 1:4 dilution of the initial cell suspension prior to seeding (Figure 2A). As shown in Figure 2B, the best TransFectin/DNA ratio for this cell type was 4  $\mu$ l/2  $\mu$ g. Furthermore, CPAE cells can be transfected



**Fig. 3. Effect of incubation time on transfection efficiency of CPAE and bovine oviductal cells.** CPAE cells ( $5 \times 10^4/\text{ml}$ ) were transfected with 1  $\mu\text{g}$  DNA (pTAL-SEAP2-control) using 1.5  $\mu\text{l}$  TransFectin, and bovine oviductal cells ( $3\text{--}5 \times 10^4/\text{ml}$ ) with 2  $\mu\text{g}$  DNA and 4  $\mu\text{l}$  TransFectin. Medium was replaced after indicated incubation times of cells with TransFectin-DNA complexes. Cell culture supernatant was assayed 24 hr (CPAE cells) or 48 hr after transfection (oviductal cells) for SEAP activity (error bars indicate standard deviation;  $n = 3$ ).



**Fig. 4. Expression of  $\beta$ -galactosidase.** Left,  $3\text{--}5 \times 10^4/\text{ml}$  bovine oviductal cells transfected with pCMV $\beta$ -Gal using 2  $\mu\text{g}$  DNA and 4  $\mu\text{l}$  TransFectin; right, CPAE cells transfected using 1  $\mu\text{g}$  DNA and 1.5  $\mu\text{l}$  TransFectin.  $\beta$ -Galactosidase activity was visualized 48 hr after transfection by histochemical staining.

efficiently using a cell density of  $5 \times 10^4/\text{ml}$  (Figure 2C). Comparable results were obtained with 1–1.5  $\mu\text{l}$  TransFectin and 1–2  $\mu\text{g}$  DNA (Figure 2D). This cell line was very sensitive to TransFectin-DNA complexes. In contrast to primary bovine oviductal cells, the removal of the complexes 2 hr after transfection was essential for CPAE cells (Figure 3). Additionally, a vector containing a  $\beta$ -galactosidase reporter gene under the control of the CMV promoter was introduced and confirmed our previous results (Figure 4).

**Table 1. Recommended transfection conditions.**

	PBMCs	BL3.1	Oviductal Cells	CPAE
Cell concentration	$4 \times 10^6/\text{ml}$	$5\text{--}7 \times 10^5/\text{ml}$	$3\text{--}5 \times 10^4/\text{ml}$	$5 \times 10^4/\text{ml}$
TransFectin/DNA ratio	4 $\mu\text{l}/2 \mu\text{g}$	7 $\mu\text{l}/2 \mu\text{g}$	4 $\mu\text{l}/2 \mu\text{g}$	1–1.5 $\mu\text{l}/1\text{--}2 \mu\text{g}$
Addition or replacement of medium after transfection	Add after 4 hr	Add after 12 hr	Not needed	Replace after 2 hr
Duration of transfection	24 hr	24 hr	48 hr	24 hr

## Conclusions

We evaluated transfection efficiency of bovine PBMCs, bovine B-lymphosarcoma cells, calf pulmonary aortic endothelial cells, and primary bovine oviductal cells using TransFectin lipid reagent. For the cell types tested, we recommend using a 24-well plate format under the transfection conditions detailed in Table 1. Remarkable transfection efficiencies were achieved by adjusting cell densities prior to transfection; quantities of TransFectin reagent and DNA; and incubation time of cells with TransFectin-DNA complexes. The results demonstrate the need for testing broad ranges of TransFectin/DNA ratios with each cell line in order to achieve maximum transfection efficiencies. TransFectin turned out to be highly efficient in these cell types, which are considered difficult to transfect.

## References

- Anderson MD et al., Transformation studies with a human T-cell leukemia virus type 1 molecular clone, *J Virol Methods* 116, 195–202 (2004)
- Fenton M et al., The efficient and rapid import of a peptide into primary B and T lymphocytes and a lymphoblastoid cell line, *J Immunol Methods* 212, 41–48 (1998)
- Remy JS et al., Gene transfer with a series of lipophilic DNA-binding molecules, *Bioconjug Chem* 5, 647–654 (1994)
- Ulbrich SE et al., Expression and localization of estrogen receptor alpha, estrogen receptor beta and progesterone receptor in the bovine oviduct in vivo and in vitro, *J Steroid Biochem Mol Biol* 84, 279–289 (2003)
- Zhou X and Huang L, DNA transfection mediated by cationic liposomes containing lipopolylysine: characterization and mechanism of action, *Biochim Biophys Acta* 1189, 195–203 (1994)
- Information in this tech note was current as of the date of writing (2004) and not necessarily the date this version (Rev A, 2005) was published.

**BIO-RAD**

**Bio-Rad  
Laboratories, Inc.**

Life Science  
Group

Web site [www.bio-rad.com](http://www.bio-rad.com) USA (800) 4BIORAD Australia 02 9914 2800 Austria (01)-877 89 01 Belgium 09-385 55 11 Brazil 55 21 2527 3454 Canada (905) 712-2771 China (86 21) 6426 0808 Czech Republic + 420 2 41 43 05 32 Denmark 44 52 10 00 Finland 09 804 22 00 France 01 47 95 69 65 Germany 089 318 84-0 Greece 30 210 777 4396 Hong Kong (852) 2789 3300 Hungary 36 1 455 8800 India (91-124)-2398112/3/4, 5018111, 6450092/93 Israel 03 951 4127 Italy 39 02 216091 Japan 03-5811-6270 Korea 82-2-3473-4460 Latin America 305-894-5950 Mexico 55-52-00-05-20 The Netherlands 0318-540666 New Zealand 64 9 415 2280 Norway 23 38 41 30 Poland + 48 22 331 99 99 Portugal 351-21-472-7700 Russia 7 095 721 1404 Singapore 65-64153188 South Africa 00 27 11 4428508 Spain 34 91 590 52 00 Sweden 08 555 12700 Switzerland 061 717 95 55 Taiwan (886 2) 2578 7189/2578 7241 United Kingdom 020 8328 2000