Transfection Protocol for Human Umbilical Vein Endothelial Cells (HUVEC) and Human Microvascular Endothelial Cells (HMVEC) in Targefect Handbook of Transfection Protocols

Rampyari Walia (targsys@aol.com)
Targefect Transfection Group, Targeting Systems

Method Article

Keywords: Targefect Transfection Protocols, HUVEC, Human Umbilical Vein Endothelial Cells, HMVECs , Human Microvascular Endothelial Cells

Posted Date: May 12th, 2011

DOI: https://doi.org/10.1038/protex.2011.225

Abstract

Efficient gene transfer into primary endothelial cells is an important prerequisite for studying regulation of gene expression in vascular tissue. Here we present a simple set of protocols using the Targefect-HUVEC ^{TM} transfection kit to transfect HUVECs, HMVECs, BAECs and different types of primary endothelial cells. The kit consists of Targefect F-2 , a non-lipid cationic transfection reagent with low toxicity along with two enhancer formulations- Virofect ^{TM} and a Peptide Enhancer. Virofect enhances gene transfer by using adenoviral receptors on the cell surface to enhance intracellular delivery of transfection complexes. Once the transfection complex is internalized, Virofect helps the transfection complex escape degradation in the lysosome and enhances the both duration and efficiency of transgene expression. The Peptide Enhancer increases efficiency of transgene expression by escorting genes to the nucleus. Using the protocols mentioned here researchers have been able to achieve 70-80% transfection efficiencies in HUVECs and efficiencies ranging form 70-80% in human and bovine lung microvascular cells \(see attached figures showing data from other lab groups and a list of product citations "http://www.targetingsystems.net/pdf/Targefect_HUVEC.pdf":http://www.targetingsystems.net/pdf/Targefect_HUVEC.pdf)

Procedure

Transfection Protocol: There are two transfection protocols for transfecting endothelial cells, one protocol uses the Targefect-HUVEC \(Targefect F-2) reagent along with the VIrofect enhancer and is used for transfecting cells in presence of serum. These protocls are applicable The second protocol uses the Targefect -HUVEC \(Targefect F-2) reagent along with the Peptide enahncer and requires that cell be transfected in the absence fo serum. Both protocols work well and there is lab to lab variation as to which one is preferred so we really suggest tryign both procols. For transfecting HUVECs, BAECs and microvascular endothelial cells using the Vriofect enhancer, we have noticed that endothelial cells should be grown in media containing at least 10% serum. **General considerations:**: Use early passage endothelial cells, avoid using collagen coated dishes as these may lower transfection efficiency. Culture media we recommend is Media 199 with 20% serum or EBM \(Cambrex) 10% FCS \(Gibco) & supplements \(Cambrex). When using any low serum media we strongly recommned increasing serum concentraiton to 10% when using the Virofect enhancer. **Cell seeding**: Set up cells so that they are approx 70% confluent at the time of experiment Preparation of the complexes and transfection procedure: Since reagents sometimes freeze during shipping, we recommend gently mixing the Targefect-F2 solution once upon receipt. The Targefect-F-2 reagent should be stored at 4 oC. Do not vortex the Targefect-HUVEC reagent. The Peptide Enhancer can be stored at 4 ^{o}C. The Virofect Enhancer should be stored at -20^{o}C or -70^{o}C. See figure in Figures section. 1| Add DMEM first. Add DNA, mix well by flicking the tube about 12 times to create a vortexing action. 2| Add Targefect next, mix well again by flicking the tube. Incubate the tubes at 37^{o}C for 25 minutes to form the transfection complexes. Condition A above is for transfecting cells in the presence of serum according to our fast protocol.-250 µl of transfection complex is added to 1 ml of complete media \(with serum) per well of a six-well dish. The dish is swirled to enable mixing of the transfection complex with the cell culture medium and the cells are incubated at 37 ^{o}C overnight and assayed for gene expression 36-48 hrs post transfection. Add 0.5 ml of transfection complex to 2 ml of complete media with serum for one 60 mm dish. For transfecting cells in a 6-well dish add 0.25 ml of transfection complex to 1 ml of complete media, for a 12-well dish add 0.125 ml transfection complex to 0.5 ml of complete media. Swirl the dish to gently mix transfection complexes with the cell culture media. Incubate overnight. Assay at 24-48 hrs after transfection. Conditions B and C are for transfecting HUVECs or HMVECs in the absence of serum 1| Aspirate cell culture media completely and add transfection complexes to the cells. The amount of transfection complexes recommended for different size dishes are shown in Table 2 below. In general the amount of transfection complex added should be just sufficient to cover the cells well for a 3 hour incubation period so that the cells dont dry up. See figure in Figures section. 2| Incubate cells with transfection complexes at 37 ^{o}C for 3 hours. Aspirate complexes and add complete media with at

least 10% serum. If you are culturing HUVECs in serum-free media, we recommend adding 10% serum to media being added immediately after aspiration of transfection complexes as this helps cells recover faster. You can change media after a few hours or the next day. Replace the media with fresh complete media the next morning and assay at 36-48 hours post-transfection. **Additional Protocols for transfecting different types of endothelial cells taken form our customer citations**: **Transfection of pulmonary microvascular endothelial cell monolayers \(PMEM) using Targefect F-2 \(Targefect-HUVEC) and Virofect)** This transfection protocol below and the pictures of tansfected lung microvascular endothelial cells has been taken form a publication from the Arnold Johnson \(Gertzberg et al, 2007) lab cited below. Pulmonary Microvessel Endothelial Cell Culture: Bovine lung microvessel endothelial cells \(BLMVEC) were obtained at passage 4 \(Vec Technologies, Rensselaer, NY; Ref. 6). The preparations were identified by Vec Technologies as pure populations by: 1) the characteristic "cobblestone" appearance as assessed by phase contrast microscopy, 2) the presence of factor VIII-related antigen, 3) the uptake of acylated low-density lipoproteins, and 4) the absence of smooth muscle actin. For all studies, BLMVEC were cultured from 4 to 12 passages in medium containing DMEM \(Gibco BRL, Grand Island, NY) supplemented with 20% fetal bovine serum \(FBS; Hyclone, Logan, UT), 15 µg/ml endothelial cell growth supplement \(Upstate Biotechnology, Lake Placid, NY), and 1% nonessential amino acids \(Gibco BRL). The BLMVEC were maintained in 5% CO2 + humidified air at 37°C. A confluent pulmonary microvessel endothelial cell monolayer \(PMEM) was reached within 2–3 population doublings, which took 3–4 days. **Transfection**. Transfection complex was formed by adding Targefect F2 \(a nonlipid cationic polymer; Targeting Systems, Santee, CA), Virofect \(an adenovirus-derived formulation to enhance transfection efficiency; Targeting Systems), and the enhanced yellow fluorescent protein \(EYFP-beta-actin) plasmids to serum-free DMEM. The transfection efficiency was similar among the groups such as in the wild-type EYFP-beta-actin \(78.0% ± 0.02%) and the mutant Y198F isoform \(79.9% ± 0.06%). The volume of reagents was formulated to achieve a final ratio of 1 µg of DNA to \(2 µl of Targefect F2 + 5 µl/ml Virofect) to provide treatment concentrations of 0.25–0.35 µg of DNA/ml of media. The complexes were incubated in 5% CO2 + humidified air at 37°C for 25 min and then added to subconfluent PMEM. After 2 h, the complex and media were removed and replaced with normal growth medium, and the cells were incubated for 24 h until confluent. Assay of Endothelial Permeability Transfection of PMEM. The BLMVEC \(0.7 x 105 in 0.5 ml of DMEM) were plated on collagen-coated Transwell-COL permeable supports \(12 mm diameter, 0.4 µm pore size; Corning, Corning, NY) and incubated for 24 h \(37°C, 5% CO2). The cells were transfected as described above adding 500 µl of the transfection complex to the top \(i.e., luminal) well and 1.5 ml of serum-free DMEM to the bottom well \(i.e., abluminal) to eliminate any hydrostatic pressure differential Citation for transfection protocol of lung microvascular endothelial cells with targefect F-2 and Vriofect Nancy Gertzberg,1,2 Tina Gurnani,3 Paul Neumann,1,2 Anne-Kay Forbes,4 Natacha Jean-Louis,4 and Arnold Johnson1,2 \(2007) Tumor necrosis factor-\{alpha} causes barrier dysfunction mediated by tyrosine198 and tyrosine218 in beta-actin Am J Physiol Lung Cell Mol Physiol 293: L1219-L1229, 2007; Transfection of BAECs, Pulmonary aortic endothelial cells: we recommend using the Targefect F-2 plus Virofect protocol described above

Timing

Transfection time varies from 30 minutes to 3 hours depending on the protocol.

Troubleshooting

Toxicity issues: Toxicity may be observed if using very low cell densities during transfection. Please use cells that are at least 60% confluent. Low cell densities nto only increase toxicity but also reduce transfection efficiency It is important to maintain cells endothelial cells in media with 10% serum during trasnfection if using Targefect F-2 plus VIrofect protocol.. This is because low serum concentraion in the media somehow results in an increse in toxicity if using this protocl. The cells are ok if switched to low serum media the day after transfection. Also, heparin in the media seems to interfere with the enhancing effect of Vrifoect and serum components reduce the interference. Cells should always be transfected in the absence of serum when usign the Targefect F-2 plus peptide enhancer protocol. This is because the

peptide enhancer works only in the absence of serum. In case toxicity is observed, aspirate trasnfection complexes after 3 hrs and add complete media to the cells

Anticipated Results

Based on our own results and customer feedback the following transfection efficiencies should be anticipated in different cell types Transfection efficiencies achieved using the Targefect- HUVEC kit: Cell types, Transfection efficiency \(%) HUVECs \(human umbilical vein endothelial cells) 70%-80% Human dermal microvascular endothelial cells 30-40% Human lung microvascular endothelial cells 70%-90% Human aortic endothelail cells 50% Bovine aortic endothelial cells 60% Porcine endothelial cells 60% Rat endothelial cells 60%

References

Selected citations are listed below A more complete list of citations \(over 30) can be found on the Taregfect-HUVEC page in " Targefect Handbook of Transfection Reagents and Protocols" at the following link http://pothi.com/pothi/book/ebook-rampyari-walia-targefect-handbook-transfection-reagents-protocols or in the targefect references section on our website link below http://www.targetingsystems.net/citations.php# Selected References: 1. Maud Martin, Michael Potente, Veerle Janssens, Didier Vertommen, Jean-Claude Twizere, Mark H. Rider, Jozef Goris, Stefanie Dimmeler, Richard Kettmann, and Franck Dequiedt \(2008) Protein phosphatase 2A controls the activity of histone deacetylase 7 during T cell apoptosis and angiogenesisPNAS, Mar 2008; 105: 4727 - 4732. 2.Nancy Gertzberg,1,2 Tina Gurnani,3 Paul Neumann,1,2 Anne-Kay Forbes,4 Natacha Jean-Louis,4 and Arnold Johnson1,2 \(2007) Tumor necrosis factor-\{alpha} causes barrier dysfunction mediated by tyrosine198 and tyrosine218 in beta-actin Am J Physiol Lung Cell Mol Physiol 293: L1219-L1229, 2007; 3. Bon-Hun Koo, David M. Coe, Laura J. Dixon, Robert P.T. Somerville, Courtney M. Nelson, Lauren W. Wang, Mary Elizabeth Young, Daniel J. Lindner, and Suneel S. Apte \(2010) ADAMTS9 Is a Cell-Autonomously Acting, Anti-Angiogenic Metalloprotease Expressed by Microvascular Endothelial Cells Am. J. Pathol.,Mar 2010; 176: 1494 - 1504. 4. Li-Wu Qian, Jianping Xie, Fengchun Ye, and Shou-Jiang Gao \(2007)Kaposi's Sarcoma-Associated Herpesvirus Infection Promotes Invasion of Primary Human Umbilical Vein Endothelial Cells by Inducing Matrix MetalloproteinasesJ. Virol., Jul 2007; 81: 7001 - 7010. 5. Smarajit Bandyopadhyay, Mohammad Z. Ashraf, Pamela Daher, Philip H. Howe, and Paul E. DiCorleto \(2007) HOXA9 Participates in the Transcriptional Activation of E-Selectin in Endothelial Cells Mol. Cell. Biol., Jun 2007; 27: 4207 - 4216. 6. Corttrell M. Kinney, Unni M. Chandrasekharan, Lori Mavrakis, and Paul E. DiCorleto \(2008)VEGF and thrombin induce MKP-1 through distinct signaling pathways: role for MKP-1 in endothelial cell migrationAm J Physiol Cell Physiol, Jan 2008; 294: C241 - C250. 7. Guoquan Liu, Jingyan Han, Jasmina Profirovic, Elena Strekalova, Tatyana A Voyno-Yasenetskaya \(2009) Galpha13 regulates MEF2-dependent gene transcription in endothelial cells: role in angiogenesisAngiogenesis \(2009) Volume: 12, Issue: 1, Pages: 1-15 8. Arnold Johnson \(2009) TNF-induced activation of pulmonary microvessel endothelial cells: a role for GSK3β. Am J Physiol Lung Cell Mol Physiol. 2009 April; 296\(4): L700–L709.

Acknowledgements

We are very grateful to all our customers for sharing their results and experience with us and giving us permission ot include some of their results in our product brochures

Figures

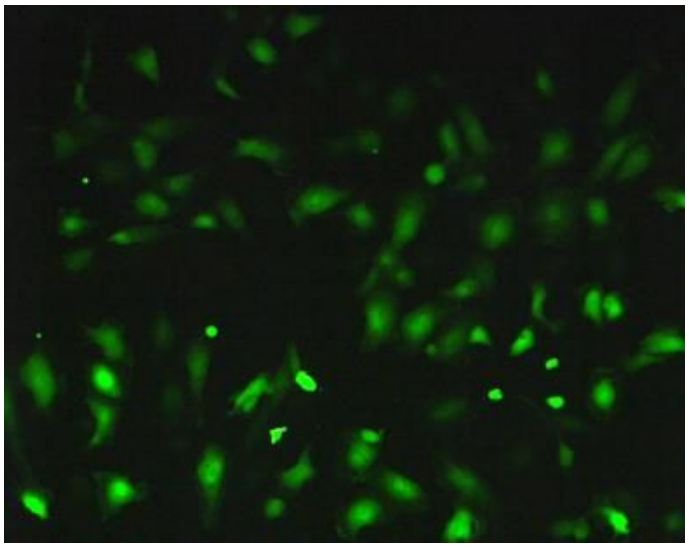

Figure 1

HUVEC2.jpg Figure 1: Transfection of primary human umbilical vein endothelial cells with a GFP expression vector using the Targefect F2 reagent plus Virofect enhancer (both components of the Targefect-HUVEC kit): 6 ug of DNA was complexed with 12 ul of Taregefct-HUVEC and 25 ul of Virofect in 0.5 ml of high glucose DMEM , and then incubated at 37 o C for 20 mins to form trasnfection complexes. . 0.5 ml of transfection complexes were added to 2 ml of fresh EBM (Cambrex), 10% FCS (GIBCO) and supplements (Cambrex) in one 60 cm dish of HUVECs. The media the next day with 3 ml of complete media. Transfection efficiency approx. 80%. Data courtesy of Dr. Michael Potente, Department of Cardiology, University of Frankfurt, Germany.

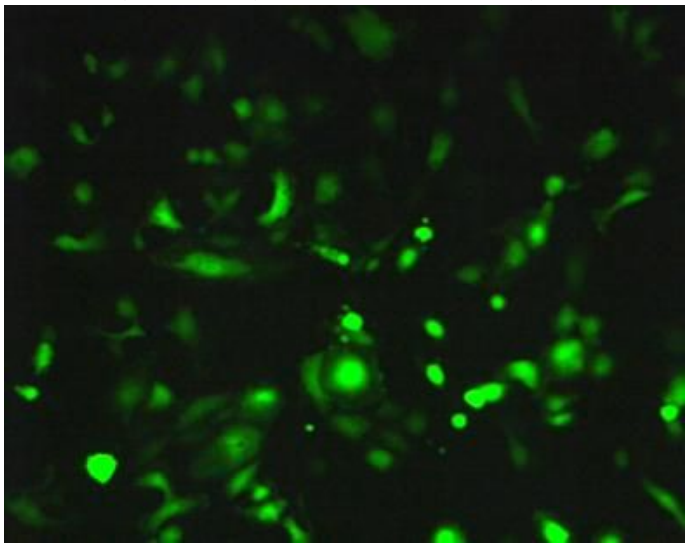

Figure 2

HUVEC transfections Figure 1:Transfection of primary human umbilical vein endothelial cells with a GFP expression vector using the Targefect F2 reagent plus Virofect enhancer (both components of the Targefect-HUVEC kit): Figure 1: Transfection of primary human umbilical vein endothelial cells with a GFP expression vector using the Targefect F2 reagent plus Virofect enhancer (both components of the Targefect-HUVEC kit): Description 6 ug of DNA was complexed with 12 ul of Taregefct-HUVEC and 25 ul of Virofect in 0.5 ml of high glucose DMEM , and then incubated at 37 o C for 20 mins to form trasnfection complexes. . 0.5 ml of transfection complexes were added to 2 ml of fresh EBM (Cambrex), 10% FCS (GIBCO) and supplements (Cambrex) in one 60 cm dish of HUVECs. The media the next day with 3 ml of

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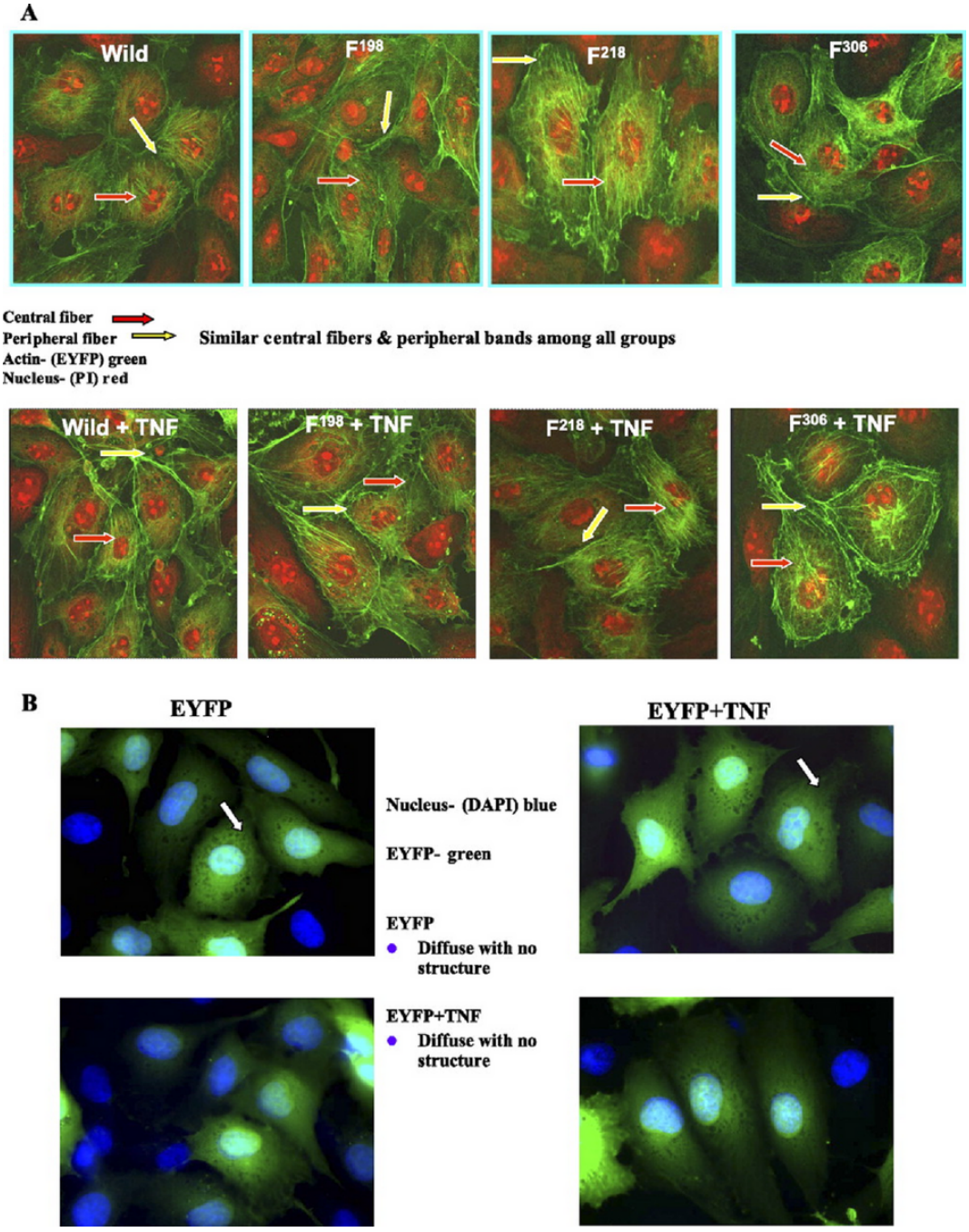

Figure 3

BLMVECtransfection-Arnold lab Transfection of (BLMVEC) bovine lung microvascular endothelial cells using Targefect F-2 (Targefect-HUVEC), and Virofect) Transfection of PMEM. The BLMVEC (0.7 x 105 in 0.5 ml of DMEM) were plated on collagen-coated Transwell-COL permeable supports (12 mm diameter, 0.4 µm pore size; Corning, Corning, NY) and incubated for 24 h (37°C, 5% CO2). The cells were transfected as described in the protocols adding 500 µl of the transfection complex to the top (i.e., luminal) well and 1.5 ml of serum-free DMEM to the bottom well (i.e., abluminal) to eliminate any hydrostatic pressure differential (see paper cited below) This figure ahs been taken form the following paper: Nancy Gertzberg,1,2 Tina Gurnani,3 Paul Neumann,1,2 Anne-Kay Forbes,4 Natacha Jean-Louis,4 and Arnold Johnson1,2 (2007) Tumor necrosis factor-{alpha} causes barrier dysfunction mediated by tyrosine198 and tyrosine218 in beta-actin Am J Physiol Lung Cell Mol Physiol 293: L1219-L1229, 2007;

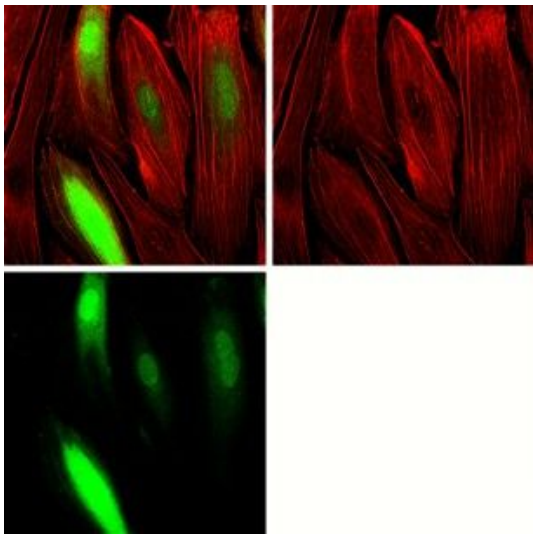

Figure 4

HMVEC transfection-Dr Duffy Figure 2: Transfection of micro vascular endothelial cells (human) with a green fluorescent protein expression vector using Targefect-HUVEC and the Peptide enhancer: Confocal images of cells transfected with a GFP-expression vector and counter-stained with rhodamine-phalloidin (actin stain) (Data courtesy of Dr. Steve Duffy and J. Murphy, UT, Southwestern Medical Ctr., Dallas, TX)

Tube #	High glucose DMEM (serum free)	DNA	Targefect	Enhancer reagent
A	0.5 ml	6 µg	12 µl F-2	25 µl Virofect
B	1 ml	1 µg	5 µl Targefect	15 µl Peptide enhancer
C	1 ml	200 ng	5 µl Targefect	10 µl Peptide enhancer

Figure 5

Table 1 In-text table

Culture Vessel	Total volume of complexes per well
24-well	0.15 ml
12-well	0.3 ml
6-well/35 mm	1 ml
60 mm	2 ml
100 mm	4 ml
150 mm	6 ml
96-well	0.04 ml

Figure 6

Table 2 In-text table 2