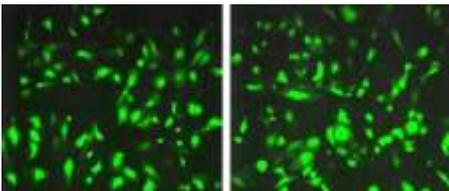


## Transfection of human endothelial cells with DNA or siRNA using Targefect reagents:

Use Targefect-HUVEC (HUVEC-01) for gene delivery

Use Targefect siRNA kit (#0060) or Targefect F-2 plus Virofect for siRNA delivery

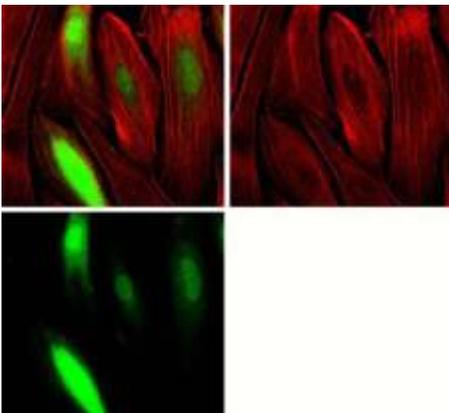
### Gene delivery into HUVECs using Targefect-HUVEC:



**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 µg of DNA was complexed with 12 µl of Targefect-HUVEC and 25 µl of Virofect in 0.5 ml of high glucose DMEM. 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. Replace the media the next day with 3 ml of complete media. Transfection efficiency is approx. 80%. **Data courtesy of Dr. Michael Potente, Department of Cardiology, University of Frankfurt, Germany.**



**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 (actinstain) (Data courtesy of Dr. Steve Duffy and J. Murphy, UT, Southwestern Medical Ctr., Dallas, TX)

## Transfection Protocols:

**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).**

**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 4oC. The Virofect Enhancer should be stored at -20oC or -70oC.

Tube #	High Glucose DMEM (Serum free)	DNA	Targefect	Enhancer reagent
1	0.5 ml	6 µg	12 µl F-2	25 µl Virofect
2	1 ml	1 µg	5 µl Targefect	15 µl Peptide enhancer
3	1 ml	200 ng	5 µl Targefect	10 µl Peptide enhancer

Add DMEM first. Add DNA, mix well by flicking the tube about 12 times to create a vortexing action. Add Targefect next, mix well again by flicking the tube. Incubate the tubes at 37oC for 25 minutes to form the transfection complexes.

Condition 1 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 oC 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 2 and 3** are for transfecting HUVECs in the absence of serum.

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

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

Incubate cells with transfection complexes at 37 °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.

## Transfection efficiencies achieved using the Targefect- HUVEC kit:

Cell types	Transfection efficiency
HUVECs (human umbilical vein endothelial cells)	70%-90%
Human dermal microvascular endothelial cells	30-40%
Human lung microvascular endothelial cells	70%-90%
Human aortic endothelial cells	50%
Bovine aortic endothelial cells	60%
Porcine endothelial cells	60%
Rat endothelial cells	60%
Various endothelial cell lines	40-90%

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