CELL
APPLICATIONS, INC.

Cytofect™-HUVEC Transfection Kit

The Cytofect™-HUVEC Transfection Kit (Cat. No. TF200K) is a plasmid DNA delivery system specifically optimized to deliver DNA into HUVEC. This kit contains Cytofect-2, Enhancer and Media for transfecting and culturing cells. The reagents in this kit are sufficient to perform 1000 reactions in 96-well format, 250 reactions in 24-well format, and 125 reactions in 12-well format. Peptide Enhancer is an endosomolytic peptide that complexes with DNA and Cytofect-2, then escorts the transfection complex to the nucleus. The results are high transfection efficiency (40-85%) and viability (80-95%) for those traditionally hard-to-transfect primary HUVEC.

I. STORAGE

- ***Open the package immediately upon arrival and examine each component for shipping damage. Notify Cell Applications, Inc. or your distributor immediately if there is any problem.***

Store the transfection reagents at the following temperatures immediately upon arrival. The reagents are stable for 1 year.

Cat #	Reagent	Volume	Temp
TF52	Cytofect-2 (CF2)	125 µl	4°C
TF53	Peptide Enhancer (PE)	125 µl	4°C
211A	Antibiotics-Free Growth Medium	2 x 125 ml	4°C
TF56	Transfection Medium	50 ml	4°C

Mix each reagent well prior to use

Cat #	Reagent	Mix
TF52	Cytofect-2 (CF2)	Invert 10 X
TF53	Peptide Enhancer (PE)	Invert 10 X

This Cytofect-HUVEC Transfection Kit contains all the necessay reagents and media for transfection. Optimal transfection protocols are provided in the kit to carry out successful transfection of the HUVEC with high efficiency (45%-65%) and viability (85%-95%).

II. PREPARATION OF HUVEC FOR TRANSFECTION

- ***Do not use freshly thawed cells for transfection. Cells must be passaged at least once prior to transfection.***
- ***Do not use cells that have been passaged more than 3 times prior to transfection.***
- ***Cells that have grown too crowded or sparse will yield poor results. Cell density at 75% confluence will yield the best transfection efficiency.***

1. Plate HUVEC (Human Umbilical Vein Endothelial Cells) at a density of 25,000 cells per cm^2 in the Antibiotics-Free Growth Medium
2. Allow cells to grow overnight. When cell density reaches 70-80% confluency next day, the culture is ready for transfection.

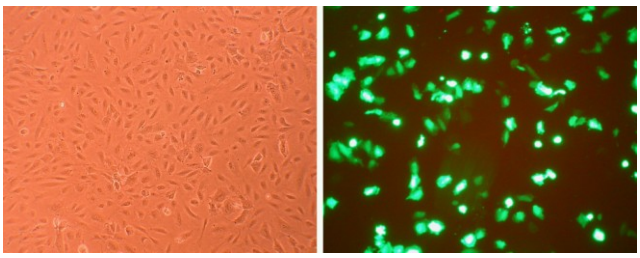

Figure 1: Transfection of HUVEC (200-05n) with a GFP-expression plasmid using CF2 with PE.

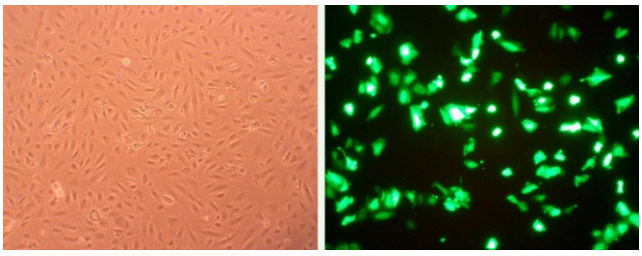

Figure 2: Transfection of HUVEC (200-05n) with GFP-expression plasmid using CF2 with PE.

III. FORMATION OF TRANSFECTION COMPLEX (Table 1)

- ***Use high purity endotoxin-free DNA for transfection.***
- ***When purifying DNA, do not overload DNA purification columns with overgrown bacterial culture preparations; consult your DNA purification instructions.***
- ***Use a positive control to confirm transfection efficiency and determine the best time to carry out experiments on the transfected cells.***

A. Prepare DNA (Step 1)

1. Dilute the plasmid DNA with Transfection Medium.
2. Mix thoroughly by flicking the tube 10 times.

B. Prepare Transfection Cocktail (Step 2)

1. Mix CF2 thoroughly by inverting the tube 10 times.
2. Add CF2 to the diluted DNA.
3. Mix thoroughly by gently flicking 10 times.
4. Mix PE by inverting the tube 10 times.
5. Add PE to the CF2-DNA mixture.
6. Mix Transfection Cocktail thoroughly by gently flicking 10 times.
7. Incubate the Transfection Cocktail at 37°C for 25 minutes to form the Transfection Complex.

IV. TRANSFECTION OF ENDOTHELIAL CELLS (Table 2)

- ***Do not let cells dry up in the well, work only on a few wells at a time.***
- ***Use Pre-equilibrated medium for all the medium changes.***

A. Add Transfection Complex (Step 3)

1. Gently aspirate off Antibiotics-Free Growth Medium from each well.
2. Add Transfection Complex to each well by gently pipetting the Transfection Complex along the side of the well so as not to disrupt cells.
3. Incubate cells with the Transfection Complex in 37°C, 5% CO_2 humidified incubator for 1 hour.

B. Replace Transfection Complex with Antibiotic-Free Growth Medium (Step 4)

1. Gently aspirate off the Transfection Complex from each well.
2. Gently add Antibiotics-Free Growth Medium to the transfected cells in each well.
3. Incubate the transfected cells in a 37°C, 5% CO_2 humidified incubator for 24 hours.
4. Change to Endothelial Cell Growth Medium and carry out experiment.

Table 1: Formation of TF2-PE Transfection Complex

CELL APPLICATIONS, INC.	STEP 1: Preparation of DNA			STEP 2: Preparation of Transfection Complex					
Tissue Culture Plate	**DNA (µg)**	**Transfection Medium (µl)**		**ADD CF2 (µl)**		**ADD PE (µl)**			**Total Transfection Complex (µl)**
96-well	0.18	60	***Gently* Flick 10X**	0.12	***Gently* Flick 10X**	0.12	***Gently* Flick 10X**	**37°C for 25'**	60.24
24-well	0.6	200		0.5		0.5			201
12-well	1.2	400		1.0		1.0			402
6-well	3.0	1000		2.5		2.5			1005

Table 2: Transfection of HUVEC with CF2-PE Transfection Complex

CELL APPLICATIONS, INC.	STEP 3: Addition of Transfection Complex			STEP 4: Replacement of Transfection Complex with Antibiotic-Free Growth Medium		
Tissue Culture Plate		**ADD Transfection Complex (µl)**			**ADD Antibiotic-Free Growth Medium (µl)**	
96-well	**Aspirate off Antibiotic-Free Growth Medium**	60.5	**37°C for 1 hr**	**Aspirate off Transfection Complex**	100	**37°C for 24 hrs**
24-well		201			500	
12-well		402			1000	
6-well		1005			2000	