CELL
APPLICATIONS, INC.

Cytofect™-Epithelial Cell Transfection Instructions

The Cytofect™-Epithalial Cell Transfection Kit (Cat. No. TF102K) is a plasmid DNA delivery system specifically optimized to deliver DNA into a wide variety of epithelial cells. This kit contains Cytofect-1, Enhancer, and Media for transfecting and culturing cells. The reagents in this kit are sufficient to perform 620 reactions in 96-well format, 124 reactions in 24-well format and 62 reactions in 12-well format. Viral Enhancer has the ability to complex with unmodified DNA via cationic Cytofect-1, and protect the transfected DNA from lysosomal degradation. The results are high transfection efficiency (40-70%) and viability (70-95%) for these traditionally hard-to-transfect primary epithelial cells.

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
TF51	Cytofect-1 (CF1)	250 µl	-20°C
TF54	Viral Enhancer (VE)*	500 µl	-20°C
511A	Antibiotics-Free Growth Medium	2 x 75 ml	4°C
TF56	Transfection Medium	10 ml	4°C
TF57	Transfection Medium with Serum	60 ml	4°C

CAUTION:** ***Viral Enhancer (VE) is an Adenovirus-derived formulation that does not contain replication competent virus, thus this viral enhancer is a replication-deficient Adenovirus preparation. However, do not use VE with cell lines that contain Adenovirus DNA, such as HEK293, that may result in complementation of the virus. When working with VE, practice laboratory biosafety used for standard Adenovirus procedures.

Mix/Vortex each reagent well prior to use.

Cat #	Reagent	Mix
TF51	Cytofect-1 (CF1)	Vortex 2 times
TF54	Viral Enhancer (VE)*	Invert 10 times

This Cytofect™-Epithelial Cell Transfection Kit has successfully transfected the following primary epithelial cells:

HEK (Human Epidermal Keratinocytes 102-05a,f,n)
HBEpC (Human Bronchial Epithelial Cells 502-05a,f)
HTEpC (Human Tracheal Epithelial Cells 504-05a,f)
HMEpC (Human Mammary Epithelial Cells 830-05a)
HPrEpC (Human Prostate Epithelial Cells 934-05a)
HUEpC (Human Urethral Epithelial Cells 9310-05a)

II. PREPARATION OF EPITHELIAL CELLS 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 cells at a density of 30,000 cells per cm^2 in the Antibiotics-Free Growth Medium.
2. Allow cells to grow overnight. Transfect cells when cell density reaches 70-80% confluence the next day.

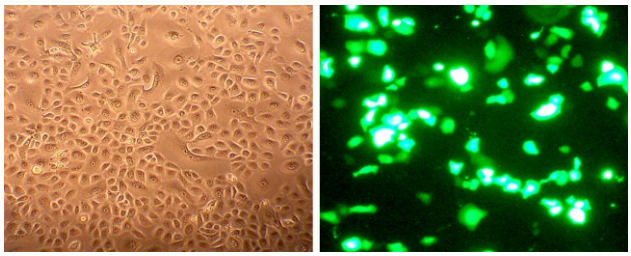

Figure 1: Transfection of HEK (Cat. No. 102K-05) with a GFP-expression plasmid using CF1 with VE*.

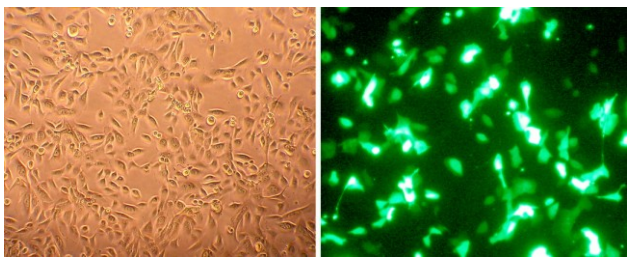

Figure 2: Transfection of HMEpC (Cat. No. 830-05) with a GFP-expression plasmid using CF1 with VE*.

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 Complex (Step 2)

1. Vortex CF1 at full speed for 30 sec. before use.
2. Add CF1 to the diluted DNA.
3. Mix thoroughly by gently flicking 10 times.
4. Mix VE* by inverting tube 10 times.
5. Add VE* to the CF1-DNA mixture.
6. Mix Transfection Cocktail thoroughly by gently flicking 12 times.
7. Incubate the Transfection Cocktail at 37°C for 20 minutes to form the Transfection Complex.
8. Add Transfection Medium with 10% serum to the Transfecton Complex.
9. Mix thoroughly by gently flicking 12 times.

IV. TRANSFECTION OF EPITHELIAL 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 3 hours.

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 Epithelial Cell Growth Medium and carry out experiment.

Table 1: Formation of CF1-VE* 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 CF1 (µl)**		**ADD VE* (µl)**			**ADD Transfection Medium with Serum (µl)**	
96-well	0.2	25	***Gently* Flick 10X**	0.4	***Gently* Flick 10Xs**	0.8	***Gently* Flick 12X**	**37°C for 20'**	100	***Gently* Flick 12X**
24-well	1.2	50		2.0		4.0			400	
12-well	2.3	150		4.0		8.0			1000	
6-well	6.0	250		12.0		24.0			2000	

Table 2: Transfection of Epithelial Cells with CF1-VE* 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**	126.2	**Incubate at 37°C 5% CO_2 for 3 hr**	**Aspirate off Transfection Complex**	100	**Incubate at 37°C 5% CO_2 for 24 hrs**
24-well		556			500	
12-well		1162			1000	
6-well		2274			2000	