CELL
APPLICATIONS, INC.

Cytofect™-Preadipocyte Transfection Instructions

The Cytofect™-Preadipocyte Cell Transfection Kit (Cat. No. TF802K) is a plasmid DNA delivery system specifically optimized to deliver DNA into Preadipocyte cells. This kit contains Cytofect-2, Enhancer, and Media for transfecting and culturing cells. The reagents in this kit are sufficient to perform 875 reactions in 96-well format, 175 reactions in 24-well format and 87 reactions in 12-well format. Viral Enhancer has the ability to complex with unmodified DNA via Cytofect-2, and protects the transfected DNA from lysosomal degradation. The results are good transfection efficiency of ~25% and viability in the range of 80-95% for these traditionally hard-to-transfect primary preadipocytes.

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)	175 µl	4°C
TF54	Viral Enhancer (VE)*	350 µl	-20°C
811A	Antibiotics-Free Growth Medium	2 x 125 ml	4°C
TF56	Transfection Medium	25 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. Do not use VE with cell lines that contain Adenovirus DNA, such as HEK293, which may result in complementation of the virus. When working with VE, practice laboratory biosafety used for standard Adenovirus procedures.

Mix each reagent well prior to use.

Cat #	Reagent	Mix
TF52	Cytofect-2 (CF2)	Invert 10 times
TF54	Viral Enhancer (VE)*	Invert 10 times

II. PREPARATION OF PREADIPOCYTE 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 15,000 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.

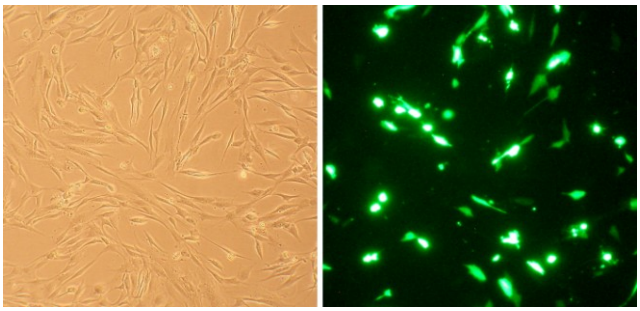

Figure: Transfection of HPAd (Cat. No. 802s-05a) with a GFP-expression plasmid using CF2 with VE*.

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. Mix CF2 by inverting the tube 10 times.
2. Add CF2 to the diluted DNA.
3. Mix thoroughly by gently flicking 10 times.
4. Mix VE* by inverting the tube 10 times.
5. Add VE* to the CF2-DNA mixture.
6. Mix Transfection Cocktail thoroughly by gently flicking 12 times.
7. Incubate the Transfection Cocktail at 37°C for 25 minutes to form the Transfection Complex.
8. Add Antibiotics-Free Growth Medium to the Transfecton Complex.
9. Mix thoroughly by gently flicking 12 times.

IV. TRANSFECTION OF PREADIPOCYTES (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 24 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 and carry out experiment.

Table 1: Formation of CF2-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 CF2 (µl)**		**ADD VE* (µl)**			**ADD Antibiotic-Free Growth Medium (µl)**	
96-well	0.06	25	***Gently* Flick 10X**	0.2	***Gently* Flick 10Xs**	0.4	***Gently* Flick 12X**	**37°C for 25'**	100	***Gently* Flick 12X**
24-well	0.2	50		1.0		2.0			400	
12-well	0.4	125		2.0		4.0			1000	
6-well	1.0	250		5.0		10.0			2000	

Table 2: Transfection of Preadipocytes with CF2-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**	125.6	**Incubate at 37°C 5% CO_2 for 24 hr**	**Aspirate off Transfection Complex**	100
24-well		453			500
12-well		1131			1000
6-well		2265			2000