CELL APPLICATIONS, INC.

Cytofect™-Fibroblast Transfection Instructions

The Cytofect™-Fibroblast Transfection Kit (Cat. No. TF103K) is a plasmid DNA delivery system specifically optimized to deliver DNA into a wide variety of fibroblasts. 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 (30-70%) and viability (90-95%) for those traditionally hard-to-transfect primary fibroblasts.

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)	250 µl	4°C
TF53	Peptide Enhancer (PE)	500 µl	4°C
106A	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 times
TF53	Peptide Enhancer (PE)	Invert 10 times

This Cytofect™-Fibroblast Transfection Kit contains all the necessary reagents and media for transfection of the following primary fibroblasts:

HDF (Human Dermal Fibroblasts 106-05a,f,n)
HCF (Human Cardiac Fibroblasts 306-05f)
HLF (Human Lung Fibroblasts 506-05a)

II. PREPARATION OF FIBROBLASTS 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 Fibroblasts at a density of 25,000 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.

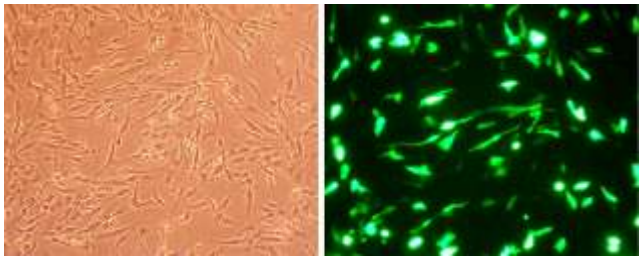

Figure 1: Transfection of HDF (Cat. No. 106-05) with a GFP-expression plasmid using CF2 with PE.

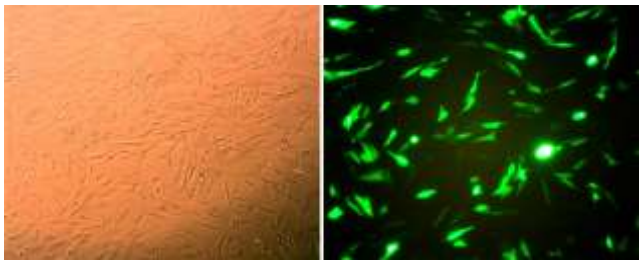

Figure 2: Transfection of HLF (Cat. No. 506-05) with a GFP-expression plasmid using CF2 with PE.

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 FIBROBLASTS (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 Fibroblast Growth Medium and carry out experiment.

Table 1: Formation of CF2-PE Transfection Complex

CELL APPLICATIONS, INC.	1. Preparation of DNA			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	0.06	60	***Gently*** **Flick 10X**	0.25	***Gently*** **Flick 10X**	0.5	***Gently*** **Flick 10X**	**37°C for 25'**	60.75
24-well	0.2	200		1.0		2.0			203
12-well	0.4	400		2.0		4.0			406
6-well	1.0	1000		5.0		10.0			1015

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

CELL APPLICATIONS, INC.	3. Addition of Transfection Complex			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**	60.75	**Incubate at 37°C 5% CO_2 for 1 hr**	**Aspirate off Transfection Complex**	100	**Incubate at 37°C 5% CO_2 for 24 hrs**
24-well		203			500	
12-well		406			1000	
6-well		1015			2000	