CELL APPLICATIONS, INC.

Cytofect™-Cell Line Transfection Instructions

The Cytofect™-Cell Line Transfection Sample Kit (Cat. No. TF104KS) is a plasmid DNA delivery system specifically optimized to deliver DNA into a wide variety of cell lines. This kit contains Cytofect-2 and Media for transfecting and culturing cells. The reagents in this kit are sufficient to perform 100 reactions in 96-well format, 25 reactions in 24-well format, and 12 reactions in 12-well format. The results are high transfection efficiency (40-90%) and viability (80-95%) for cell lines.

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)	25 µl	4ºC
TF55	Antibiotics-Free Growth Medium	25 ml	4ºC
TF56	Transfection Medium	5 ml	4ºC

Mix each reagent well prior to use.

Cat #	Reagent	Mix
TF52	Cytofect-2 (CF2)	Invert 10 times

The Cytofect™-Cell Line Transfection Kit contains all the necessay reagents and media for transfection of the following cell lines:

CHO
HEK293
HeLa
MCF7

II. PREPARATION OF CELLS FOR TRANSFECTION

- ***Do not use freshly thawed cells for transfection. Cells must be passaged at least once 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 in the Antibiotics-Free Growth Medium such that 70%-80% confluency will be reached the next day. The seeding density will vary depending on specific cell line.
2. Allow cells to grow overnight. Transfect cells when cell density reaches 70-80% confluence the next day.

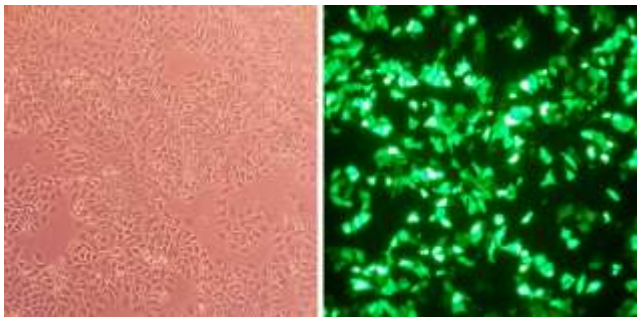

Figure 1: Transfection of MCF-7 cells with a GFP-expression plasmid using CF2

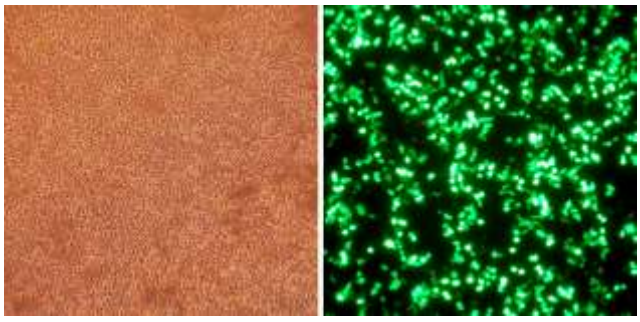

Figure 2: Transfection of HEK293 cells with a GFP-expression plasmid using CF2.

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 tube 10 times.
2. Add CF2 to the diluted DNA.
3. Mix thoroughly by gently flicking 10 times.
4. Incubate the Transfection Cocktail at 37°C for 25 minutes.

IV. TRANSFECTION OF 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. Aspirate off Antibiotics-Free Growth Media from cell culture.
2. Add the appropriate amount of 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 Growth Medium and carry out experiment.

Table 1: Formation of CF2 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)**			**Total Transfection Complex (µl)**
96-well	0.06	60	***Gently*** **Flick 10X**	0.25	***Gently*** **Flick 10X**	**37°C for 25'**	60.25
24-well	0.2	200		1.0			201
12-well	0.4	400		2.0			402
6-well	1.0	1000		5.0			1005

Table 2: Transfection of Cells with CF2 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**	60.25	**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		201			500	
12-well		402			1000	
6-well		1005			2000	