CELL APPLICATIONS, INC.

Cytofect™ Neuron Transfection Instructions

The Cytofect™ NeuronTransfection Kit (Cat. No. TF886K) is a plasmid DNA delivery system specifically optimized to deliver DNA into neuron cells. This kit contains Cytofect-1, Enhancer and Media for transfecting and culturing cells. The reagents in this kit are sufficient to perform 340 reactions in 96-well format, 100 reactions in 24-well format, and 50 reactions in 12-well format. Peptide Enhancer is an endosomolytic peptide that complexes with DNA and Cytofect-1, then escorts the transfection complex to the nucleus. The results are high transfection efficiency (10-20%) and viability (80-95%) for those traditionally hard-to-transfect primary neuron 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	-20ºC
TF53	Peptide Enhancer (PE)	500 µl	4ºC
817A	Antibiotics-Free Growth Medium	125 ml	4ºC
TF56	Transfection Medium	25 ml	4ºC

Mix/Vortex each reagent well prior to use

Cat #	Reagent	Mix
TF51	Cytofect-1 (CF1)	Vortex 2 X
TF53	Peptide Enhancer (PE)	Invert 10 X

This Cytofect-Neuron Transfection Kit has successfully transfected the following primary neuron cells with an efficiency of 11% and viability (70%-95%).

RHiN (Rat Hippocampal Neurons)
RCON (Rat Cortical Neurons)

II. PREPARATION OF NEURONS 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 Neurons at a density of about 50,000 cells per cm^2 in the Antibiotics-Free Growth Medium
2. Allow cells to grow for 8 days. Cell density should reach 70-80% confluency and be ready for transfection.

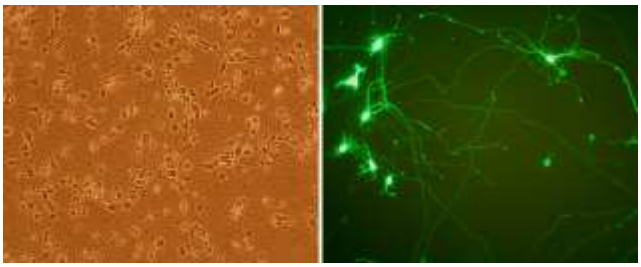

Figure 1: Transfection of RCON with a GFP-expression plasmid using CF1 with PE, 1 day post transfection.

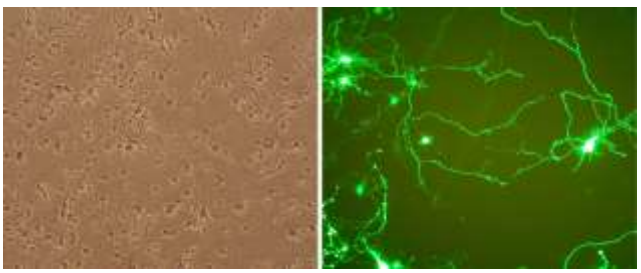

Figure 2: Transfection of RCON with a GFP-expression plasmid using CF1 with PE, 3 days post transfection.

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. Preparation of DNA (Step 1)

1. Dilute the plasmid DNA with Transfection Medium in Table 3.
2. Mix thoroughly by flicking 10 times.

B. Preparation of Transfection Complex (Step 2)

1. Vortex CF1 at full speed 2x for 30sec. before use.
2. Add CF1 to the diluted DNA as shown in Table 1.
3. Mix thoroughly by gently flicking 10 times.
4. Mix PE by inverting tube 10 times.
5. Add PE to the CF1-DNA mixture as shown in Table 3.
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.

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. Addition of 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 as shown in Table 2 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. Replacement of Transfection Complex with Antibiotic-Free Growth Medium (Step 4)

1. Aspirate off the Transfection Complex from cells.
2. Add Pre-warmed Antibiotics-Free Growth Medium to the transfected cells as shown in Table 2.
3. Incubate the transfected cells in 37°C, 5% CO2 humidified incubated for 24 hours and assay.

Table 1: Formation of CF1-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 CF1 (µl)**		**ADD PE (µl)**			**Total Transfection Complex (µl)**
96-well	0.36	60	***Gently* Flick 10X to mix**	0.72	***Gently* Flick 10X to mix**	1.5	***Gently* Flick 10X to mix**	**Incubate at 37°C for 25min**	62
24-well	1.2	200		2.4		5.0			208
12-well	2.4	400		4.8		10.0			416
6-well	6.0	1000		12.0		25.0			1040

Table 2: Transfection of Neuron Cells with CF1-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**	62	**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		208			500	
12-well		416			1000	
6-well		1040			2000	