CELL
APPLICATIONS, INC.

Cytofect™-Hepatocyte Transfection Instructions

The Cytofect™-Hepatocyte Transfection Kit (Cat. No. TF780K) is a plasmid DNA delivery system specifically optimized to deliver DNA into hepatocytes. This kit contains Cytofect-1, Enhancer and Media for transfecting and maintaining cells. The reagents in this kit are sufficient to perform 1000 reactions in 96-well format, 200 reactions in 24-well format, and 100 reactions in 12-well format. Viral Enhancer has the ability to complex with unmodified DNA via cationic Cytofect-1 to protect the transfected DNA from lysosomal degradtion. The result are high transfection efficiency (40%-60%) and viability for these traditionally hard-to-transfect primary hepatocytes (70%-95%).

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
715A	Antibiotics-Free Culture Medium	2 x 100 ml	4°C
TF56	Transfection Medium	10 ml	4°C
TF58	Antibiotic-Free Serum-Containing Medium	50 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

II. PREPARATION OF HEPATOCYTES FOR TRANSFECTION

- ***Cells that are too crowded or sparse will yield poor results. Cell density at 75% confluence will yield the best transfection efficiency.***

1. Plate Hepatocytes at a density of 62,500 cells per cm^2 in the Antibiotics-Free Culture Medium (715A).
2. Allow cells to plate overnight. Cell density should reach 70-80% confluency the next day and be ready for transfection.

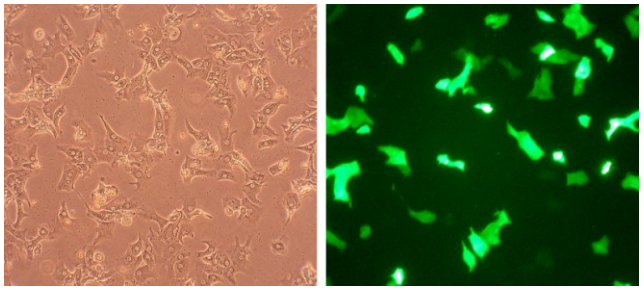

Figure 1: Transfection of Rat Hepatocytes with a GFP-expression plasmid using CF1 with VE* (10X magnification).

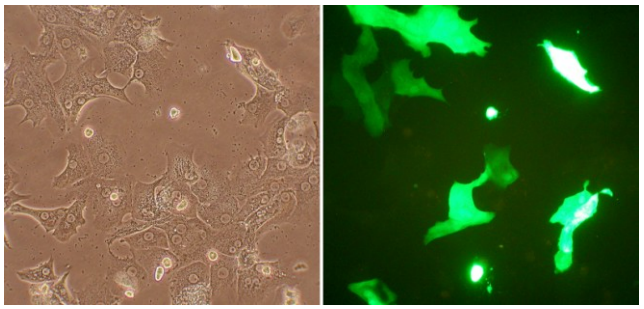

Figure 2: Transfection of Rat Hepatocytes with a GFP-expression plasmid using CF1 with VE* (20X magnification).

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.***
- ***Pre-equilibrate antibiotic-free growth medium according to the volume in Table 1, Step 2.***

A. Prepare DNA (Step 1)

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

B. Prepare Transfection Complex (Step 2)

1. Vortex CF1 at full speed 2x for 30sec. 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 the Transfection Cocktail thoroughly by gently flicking 12 times.
7. Incubate the Transfection Cocktail at 37°C for 20 minutes to form Transfection Complex.

IV. TRANSFECTION OF HEPATOCYTES (Table 2)

- ***Do not let cells dry up in the well, work only on a few wells at a time.***

A. Replace Antibiotics-Free Culture Medium with Antibiotics-Free Serum-Containing Medium. (Step 3)

1. Pre-equilibrate appropriate amount of Antibiotics-Free Serum-Containing Medium (TF58).
2. Gently aspirate off Antibiotics-Free Culture Medium (715A) from hepatocytes.
3. Add Pre-equilibrate Antibiotics-Free Serum-Containing Medium (TF58) to hepatocytes at designated volume.

B. Add Transfection Complex (Step 4)

1. 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.
2. Incubate cells with the Transfection Complex in 37°C, 5% CO_2 humidified for 24 hours.

C. Replace Transfection Complex with Antibiotics-Free Growth Medium (Step 5)

1. Gently aspirate off the Transfection Complex from each well.
2. Change to Antibiotics-Free Culture Medium and assay.

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)**		
96-well	0.09	25	***Gently*** **Flick 10X to mix**	0.25	***Gently*** **Flick 10X to mix**	0.5	***Gently*** **Flick 12X to mix**	**Incubate at 37°C for 20min**
24-well	0.3	50		1.25		2.5		
12-well	0.6	150		2.5		5.0		
6-well	1.5	250		7.5		15.0		

Table 2: Transfection of Hepatocytes with CF1-VE* Transfection Complex

CELL APPLICATIONS, INC.	Step 3: Replacement of Antibiotics-Free Culture Medium with Antibiotics-Free Serum-Containing Medium		Step 4: Addition of Transfection Complex		Step 5: Replacement of Transfecion Complex with Antibiotics-Free Culture Medium	
Tissue Culture Plate		**ADD Antibiotics-Free Serum Containing Medium (µl)**	**ADD Transfection Complex (µl)**			**ADD Antibiotic-Free Growth Medium (µl)**
96-well	**Aspirate off Antibiotics-Free Culture Medium**	30	25.75	**Incubate at 37°C 5% CO_2 for 24 hr**	**Aspirate off Transfection Complex**	100
24-well		150	53.75			500
12-well		300	157.50			1000
6-well		600	272.50			2000