CELL
APPLICATIONS, INC.

Cytofect™-Smooth Muscle Cell Transfection Instructions

The Cytofect™-Smooth Muscle Cell Transfection Kit (Cat. No. TF350K) is a plasmid DNA delivery system specifically optimized to deliver DNA into a wide variety of smooth muscle cells. This kit contains Cytofect-2 and Media for transfecting and culturing cells. The reagents in this kit are sufficient to perform 500 reactions in 96-well format, 125 reactions in 24-well format, and 62 reactions in 12-well format. The results are high transfection efficiency (40-90%) and viability (80-95%) for those traditionally hard-to-transfect primary smooth muscle 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
TF52	Cytofect-2 (CF2)	250 µl	4°C
311A	Antibiotics-Free Growth Medium	125 ml	4°C
TF56	Transfection Medium	40 ml	4°C

Mix each reagent well prior to use.

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

The Cytofect™-Smooth Muscle Cell Transfection Kit contains all the necessary reagents and media for transfection of the following cells:

HAOSMC (Human Aortic Smooth Muscle Cells 354-05a,f)
HBcASMC (Human Brachiocephalic Artery Smooth Muscle Cells 3512-05a)
HCASMC (Human Coronoary Artery Smooth Muscle Cells 350-05a)
HITASMC (Human Internal Thoracic Artery Smooth Muscle Cells 358-05a)
HPASMC (Human Pulmonary Artery Smooth Muscle Cells 352-05a)
HScASMC (Human Subclavian Artery Smooth Muscle Cells 3510-05a)

II. PREPARATION OF 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 in the Antibiotics-Free Growth Medium such that 70%-80% confluency will be reached the next day.
2. Allow cells to grow overnight. Transfect cells when cell density reaches 70-80% confluence the next day.

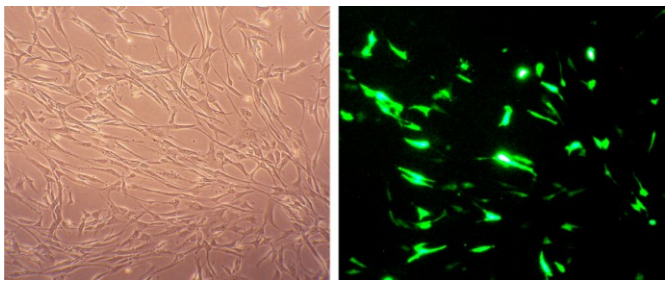

Figure 1: Transfection of HAOSMC (Cat. No. 354-05a) with a GFP-expression plasmid using CF2

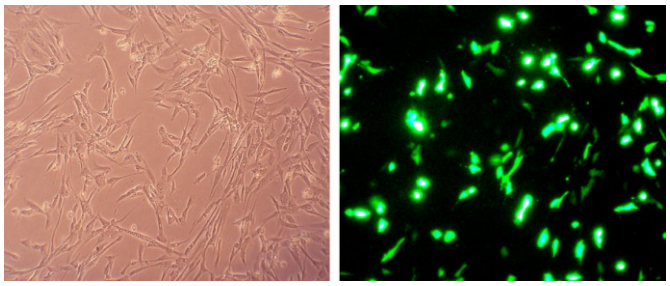

Figure 2: Transfection of HCASMC (Cat. No. 350-05a) with a GFP-expression plasmid using CF2.

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. Preparation of DNA (Step 1)

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

B. Preparation of Transfection Complex (Step 2)

1. Mix CF2 by inverting tube 10 times.
2. Add CF2 to the diluted DNA as shown in Table 1.
3. Mix the Transfection Cocktail thoroughly by gently flicking 10 times.
4. Incubate the Transfection Cocktail at 37°C for 25 minutes to form the Transfection Complex.

IV. TRANSFECTION OF SMOOTH MUSCLE CELLS

- ***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 Antibiotics-Free Growth Medium to the transfected cells as shown in Table 2.
3. Incubate the transfected cells in 37°C, 5% CO_2 humidified incubator for 24 hours.
4. Change to Growth Medium and assay.

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	0.12	60	***Gently*** **Flick 10X**	0.5	***Gently*** **Flick 10X**	**37°C for 25'**	60.5
24-well	0.4	200		2.0			202
12-well	0.8	400		4.0			404
6-well	2.0	1000		10.0			1010

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**	60.5	**37°C 5% CO_2 for 1 hr**	**Aspirate off Transfection Complex**	100	**37°C 5% CO_2 for 24 hrs**
24-well		202			500	
12-well		404			1000	
6-well		1010			2000	