CELL APPLICATIONS, INC.

Cytofect™-Smooth Muscle Cell Transfection Instructions

The Cytofect™-Smooth Muscle Cell Transfection Sample Kit (Cat. No. TF350KS) 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 50 reactions in 96-well format, 12 reactions in 24-well format, and 6 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)	25 µl	4ºC
311A	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™-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)

I. 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.

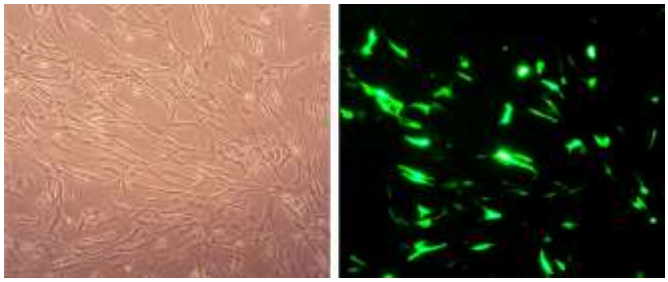

Figure 1: Transfection of HAOSMC (Cat. No. 354-05a) with a GFP-expression plasmid using CF2

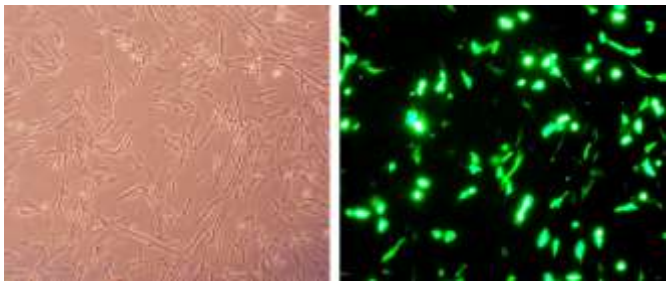

Figure 2: Transfection of HCASMC (Cat. No. 350-05a) with a GFP-expression plasmid using CF2.

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 Cocktail (Step 2)

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

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 Cocktail (Step 3)

1. Aspirate off Antibiotics-Free Growth Media from cell culture.
2. Add the appropriate amount of Transfection Cocktail to each well as shown in Table 2 by gently pipetting the Transfection Cocktail along the side of the well so as not to disrupt cells.
3. Incubate cells with the Transfection Cocktail in 37°C, 5% CO_2 humidified incubator for 1 hour.

B. Replacement of Transfection Cocktail with Antibiotic-Free Growth Medium (Step 4)

1. Aspirate off the Transfection Cocktail 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 Cocktail

CELL APPLICATIONS, INC.	STEP 1 Preparation of DNA			STEP 2 Preparation of Transfection Cocktail			
Tissue Culture Plate	**DNA (µg)**	**Transfection Medium (µl)**		**ADD CF2 (µl)**		**37°C for 25'**	**Total Transfection Cocktail (µl)**
96-well	0.12	60	***Gently*** **Flick 10X**	0.5	***Gently*** **Flick 10X**		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 Cocktail

CELL APPLICATIONS, INC.	STEP 3 Addition of Transfection Cocktail			STEP 4 Replacement of Transfection Cocktail with Antibiotic-Free Growth Medium		
Tissue Culture Plate		**ADD Transfection Cocktail (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 Cocktail**	100	**37°C 5% CO_2 for 24 hrs**
24-well		202			500	
12-well		404			1000	
6-well		1010			2000	