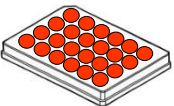
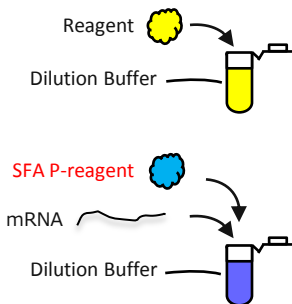

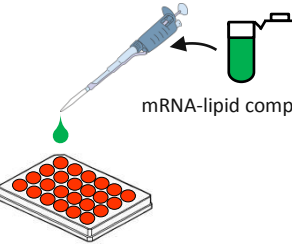
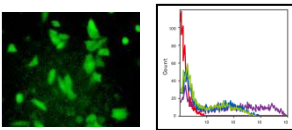


### 1-Step method (Reverse transfection method)

Timeline	Steps
<p><b>1</b></p> <p>Reagent Dilution Buffer</p> <p>SFA P-reagent mRNA Dilution Buffer</p>	<p>Dilute ScreenFect™ A plus Reagent*<sup>1</sup> in Dilution Buffer, and then mix well *<sup>1</sup> Vortex the reagent before use</p> <p>Dilute mRNA and SFA P-reagent in Dilution Buffer, and then mix well</p>
<p><b>2</b></p> <p>mRNA-lipid complex</p>	<p>Add diluted mRNA (+SFA P-reagent) to diluted ScreenFect™ A plus Reagent, and then incubate for 5 minutes ~ at room temperature*<sup>2</sup> *<sup>2</sup> Incubation is available until the step 4 has been completed</p>
<p><b>3</b></p> <p>Cultured cells</p>	<p>Prepare required cells for transfection</p>
<p><b>4</b></p> <p>Cell suspension</p>	<p>Detach cells and prepare the cell suspension, and then transfer the required numbers of cell suspension to cell culture plate</p>
<p><b>5</b></p> <p>mRNA-lipid complex</p>	<p>Add mRNA-lipid complex from step 2 to well of cell culture plate from step 4</p>
<p><b>6</b></p>	<p>Visualize/analyze transfected cells</p>

Procedure Details		96-well	24-well	12-well	6-well
Component					
Dilution Buffer for ScreenFect™ A plus		5 µL	25 µL	50 µL	125 µL
mRNA : Transfection Reagent ratio		1 : 3	1 : 4	1 : 3	1 : 4
ScreenFect™ A plus Transfection Reagent		0.15 µL	0.2 µL	0.75 µL	1.0 µL
Dilution Buffer for ScreenFect™ A plus		5 µL	25 µL	50 µL	125 µL
mRNA (0.1-2.5 µg / µL)		50 ng	250 ng	500 ng	1250 ng
SFA P-reagent (2µL / µg mRNA)		0.1 µL	0.5 µL	1.0 µL	2.5 µL
Diluted mRNA (+SFA P-reagent)		5 µL	25 µL	50 µL	125 µL
Diluted ScreenFect™ A plus Transfection Reagent		5 µL	25 µL	50 µL	125 µL
Adherent cells or suspension cells		1.0-4.0 × 10 <sup>4</sup>	0.5-2.0 × 10 <sup>5</sup>	1.0-4.0 × 10 <sup>5</sup>	0.25-1.0 × 10 <sup>6</sup>
Cell Detachment (Trypsin or Accutase®)					
Final composition [per well]					
mRNA-lipid complex		10 µL	50 µL	100 µL	250 µL
mRNA amount		50 ng	250 ng	500 ng	1250 ng
SFA P-reagent used		0.1 µL	0.5 µL	1.0 µL	2.5 µL
ScreenFect™ A plus Transfection Reagent used		0.15 or 0.2 µL	0.75 or 1.0 µL	1.5 or 2.0 µL	3.75 or 5.0 µL
Medium volume		100 µL	500 µL	1000 µL	2000 µL
Incubate cells for 1 day ~ at 37°C. Then, analyze transfected cells.					

### 2-Step method (Forward transfection method)

Timeline		Steps	Procedure Details										
1	Day 0	 Pre-Cultured cells	Seed cells to be 70-90% confluent at transfection	Component									
				96-well		24-well		12-well		6-well			
				Adherent cells or suspension cells		1.0-4.0 × 10 <sup>4</sup>		0.5-2.0 × 10 <sup>5</sup>		1.0-4.0 × 10 <sup>5</sup>		0.25-1.0 × 10 <sup>6</sup>	
				Seed cells to be 70-90% confluent at transfection. The medium replacement may improve a transfection efficiency in a 2-Step method.									
2		 Reagent Dilution Buffer SFA P-reagent mRNA Dilution Buffer	Dilute ScreenFect™ A plus Reagent*1 in Dilution Buffer, and then mix well ※1 Vortex the reagent before use  Dilute mRNA and SFA P-reagent in Dilution Buffer, and then mix well	Dilution Buffer for ScreenFect™ A plus									
				5 μL		25 μL		50 μL		125 μL			
				mRNA : Transfection Reagent ratio		1 : 3		1 : 4		1 : 3		1 : 4	
				ScreenFect™ A plus Transfection Reagent		0.15 μL		0.2 μL		0.75 μL		1.0 μL	
				Dilution Buffer for ScreenFect™ A plus		5 μL		25 μL		50 μL		125 μL	
				mRNA (0.1-2.5 μg / μL)		50 ng		250 ng		500 ng		1250 ng	
				SFA P-reagent (2μL / μg mRNA)		0.1 μL		0.5 μL		1.0 μL		2.5 μL	
3	Day 1	 mRNA-lipid complex	Add diluted mRNA (+SFA P-reagent) to diluted ScreenFect™ A plus Reagent, and then incubate for 5 minutes ~ at room temperature*2 ※2 Incubation is available until the step 4 has been completed	Diluted mRNA (+SFA P-reagent)									
				5 μL		25 μL		50 μL		125 μL			
				Diluted ScreenFect™ A plus Transfection Reagent									
				5 μL		25 μL		50 μL		125 μL		125 μL	
4		 mRNA-lipid complex	Add mRNA-lipid complex from step 3 to well of cell culture plate from step 1	Final composition [per well]									
				96-well		24-well		12-well		6-well			
				mRNA-lipid complex		10 μL		50 μL		100 μL		250 μL	
				mRNA amount		50 ng		250 ng		500 ng		1250 ng	
				SFA P-reagent used		0.1 μL		0.5 μL		1.0 μL		2.5 μL	
				ScreenFect™ A plus Transfection Reagent used		0.15 or 0.2 μL		0.75 or 1.0 μL		1.5 or 2.0 μL		3.75 or 5.0 μL	
				Medium volume		100 μL		500 μL		1000 μL		2000 μL	
5	Day 2 ~		Visualize/analyze transfected cells	Incubate cells for 1 day ~ at 37°C. Then, analyze transfected cells.									