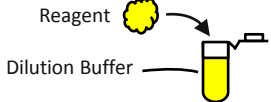
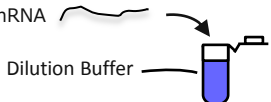
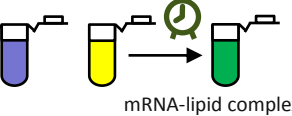

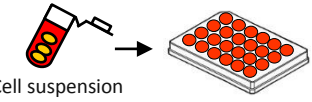
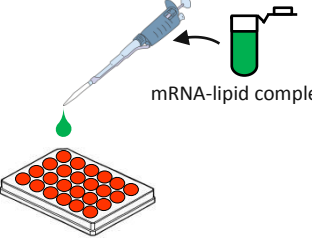
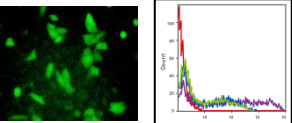
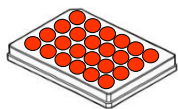
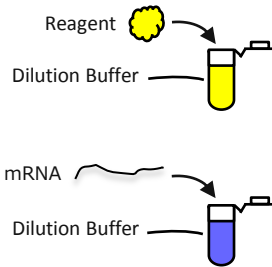

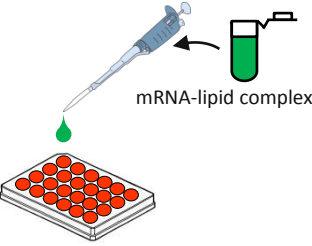
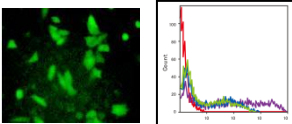


1-Step method (Reverse transfection method)

Timeline		Steps	Procedure Details																																											
Day 0	1  Reagent Dilution Buffer	Dilute ScreenFect™A plus Reagent* ¹ in Dilution Buffer, and then mix well ※1 Vortex the reagent before use	<table border="1"> <thead> <tr> <th>Component</th> <th colspan="2">96-well</th> <th colspan="2">24-well</th> <th colspan="2">12-well</th> <th colspan="2">6-well</th> </tr> </thead> <tbody> <tr> <td>Dilution Buffer for ScreenFect™A plus</td> <td colspan="2">5 μL</td> <td colspan="2">25 μL</td> <td colspan="2">50 μL</td> <td colspan="2">125 μL</td> </tr> <tr> <td>mRNA : Transfection Reagent ratio</td> <td>1 : 3</td> <td>1 : 4</td> <td>1 : 3</td> <td>1 : 4</td> <td>1 : 3</td> <td>1 : 4</td> <td>1 : 3</td> <td>1 : 4</td> </tr> <tr> <td>ScreenFect™A plus Transfection Reagent</td> <td>0.15 μL</td> <td>0.2 μL</td> <td>0.75 μL</td> <td>1.0 μL</td> <td>1.5 μL</td> <td>2.0 μL</td> <td>3.75 μL</td> <td>5.0 μL</td> </tr> </tbody> </table>								Component	96-well		24-well		12-well		6-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	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	1.5 μL	2.0 μL	3.75 μL	5.0 μL
	Component	96-well		24-well		12-well		6-well																																						
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	ScreenFect™A plus Transfection Reagent	0.15 μL	0.2 μL	0.75 μL	1.0 μL	1.5 μL	2.0 μL	3.75 μL	5.0 μL																																					
	 mRNA Dilution Buffer	Dilute mRNA in Dilution Buffer, and then mix well	<table border="1"> <tbody> <tr> <td>Dilution Buffer for ScreenFect™A plus</td> <td colspan="2">5 μL</td> <td colspan="2">25 μL</td> <td colspan="2">50 μL</td> <td colspan="2">125 μL</td> </tr> <tr> <td>mRNA (0.1-2.5 μg / μL)</td> <td colspan="2">50 ng</td> <td colspan="2">250 ng</td> <td colspan="2">500 ng</td> <td colspan="2">1250 ng</td> </tr> </tbody> </table>								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																			
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2  mRNA-lipid complex	Add diluted mRNA 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	<table border="1"> <tbody> <tr> <td>Diluted mRNA</td> <td colspan="2">5 μL</td> <td colspan="2">25 μL</td> <td colspan="2">50 μL</td> <td colspan="2">125 μL</td> </tr> <tr> <td>Diluted ScreenFect™A plus Transfection Reagent</td> <td colspan="2">5 μL</td> <td colspan="2">25 μL</td> <td colspan="2">50 μL</td> <td colspan="2">125 μL</td> </tr> </tbody> </table>								Diluted mRNA	5 μL		25 μL		50 μL		125 μL		Diluted ScreenFect™A plus Transfection Reagent	5 μL		25 μL		50 μL		125 μL																				
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3  Cultured cells	Prepare required cells for transfection	<table border="1"> <tbody> <tr> <td>Adherent cells or suspension cells</td> <td colspan="2">1.0-4.0 × 10⁴</td> <td colspan="2">0.5-2.0 × 10⁵</td> <td colspan="2">1.0-4.0 × 10⁵</td> <td colspan="2">0.25-1.0 × 10⁶</td> </tr> </tbody> </table>								Adherent cells or suspension cells	1.0-4.0 × 10 ⁴		0.5-2.0 × 10 ⁵		1.0-4.0 × 10 ⁵		0.25-1.0 × 10 ⁶																													
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4  Cell suspension	Detach cells and prepare the cell suspension, and then transfer the required numbers of cell suspension to cell culture plate	<table border="1"> <tbody> <tr> <td>Cell Detachment (Trypsin or Accutase®)</td> <td colspan="2"></td> <td colspan="2"></td> <td colspan="2"></td> <td colspan="2"></td> </tr> </tbody> </table>								Cell Detachment (Trypsin or Accutase®)																																				
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5  mRNA-lipid complex	Add mRNA-lipid complex from step 2 to well of cell culture plate from step 4	<table border="1"> <thead> <tr> <th>Final composition [per well]</th> <th>96-well</th> <th>24-well</th> <th>12-well</th> <th>6-well</th> </tr> </thead> <tbody> <tr> <td>mRNA-lipid complex</td> <td>10 μL</td> <td>50 μL</td> <td>100 μL</td> <td>250 μL</td> </tr> <tr> <td>mRNA amount</td> <td>50 ng</td> <td>250 ng</td> <td>500 ng</td> <td>1250 ng</td> </tr> <tr> <td>ScreenFect™A plus Transfection Reagent used</td> <td>0.15 or 0.2 μL</td> <td>0.75 or 1.0 μL</td> <td>1.5 or 2.0 μL</td> <td>3.75 or 5.0 μL</td> </tr> <tr> <td>Medium volume</td> <td>100 μL</td> <td>500 μL</td> <td>1000 μL</td> <td>2000 μL</td> </tr> </tbody> </table>								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	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												
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6 Day 1 ~ 	Visualize/analyze transfected cells	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	Seed cells to be 70-90% confluent at transfection. The medium replacement may improve a transfection efficiency in a 2-Step method.																																																						
				<table border="1"> <thead> <tr> <th>Component</th> <th colspan="2">96-well</th> <th colspan="2">24-well</th> <th colspan="2">12-well</th> <th colspan="2">6-well</th> </tr> </thead> <tbody> <tr> <td>Adherent cells or suspension cells</td> <td colspan="2">1.0-4.0 × 10⁴</td> <td colspan="2">0.5-2.0 × 10⁵</td> <td colspan="2">1.0-4.0 × 10⁵</td> <td colspan="2">0.25-1.0 × 10⁶</td> </tr> </tbody> </table>										Component	96-well		24-well		12-well		6-well		Adherent cells or suspension cells	1.0-4.0 × 10 ⁴		0.5-2.0 × 10 ⁵		1.0-4.0 × 10 ⁵		0.25-1.0 × 10 ⁶																												
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2		 Reagent Dilution Buffer mRNA Dilution Buffer	Dilute ScreenFect™A plus Reagent^{※1} in Dilution Buffer, and then mix well ^{※1} Vortex the reagent before use Dilute mRNA in Dilution Buffer, and then mix well	<table border="1"> <tbody> <tr> <td>Dilution Buffer for ScreenFect™A plus</td> <td colspan="2">5 μL</td> <td colspan="2">25 μL</td> <td colspan="2">50 μL</td> <td colspan="2">125 μL</td> </tr> <tr> <td>mRNA : Transfection Reagent ratio</td> <td>1 : 3</td> <td>1 : 4</td> <td>1 : 3</td> <td>1 : 4</td> <td>1 : 3</td> <td>1 : 4</td> <td>1 : 3</td> <td>1 : 4</td> </tr> <tr> <td>ScreenFect™A plus Transfection Reagent</td> <td>0.15 μL</td> <td>0.2 μL</td> <td>0.75 μL</td> <td>1.0 μL</td> <td>1.5 μL</td> <td>2.0 μL</td> <td>3.75 μL</td> <td>5.0 μL</td> </tr> </tbody> </table>										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	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	1.5 μL	2.0 μL	3.75 μL	5.0 μL																		
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4		 mRNA-lipid complex	Add mRNA-lipid complex from step 3 to well of cell culture plate from step 1	<table border="1"> <thead> <tr> <th>Final composition [per well]</th> <th colspan="2">96-well</th> <th colspan="2">24-well</th> <th colspan="2">12-well</th> <th colspan="2">6-well</th> </tr> </thead> <tbody> <tr> <td>mRNA-lipid complex</td> <td colspan="2">10 μL</td> <td colspan="2">50 μL</td> <td colspan="2">100 μL</td> <td colspan="2">250 μL</td> </tr> <tr> <td>mRNA amount</td> <td colspan="2">50 ng</td> <td colspan="2">250 ng</td> <td colspan="2">500 ng</td> <td colspan="2">1250 ng</td> </tr> <tr> <td>ScreenFect™A plus Transfection Reagent used</td> <td colspan="2">0.15 or 0.2 μL</td> <td colspan="2">0.75 or 1.0 μL</td> <td colspan="2">1.5 or 2.0 μL</td> <td colspan="2">3.75 or 5.0 μL</td> </tr> <tr> <td>Medium volume</td> <td colspan="2">100 μL</td> <td colspan="2">500 μL</td> <td colspan="2">1000 μL</td> <td colspan="2">2000 μL</td> </tr> </tbody> </table>										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		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	
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