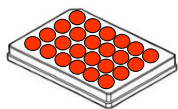
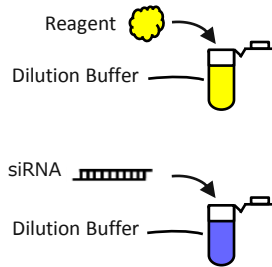

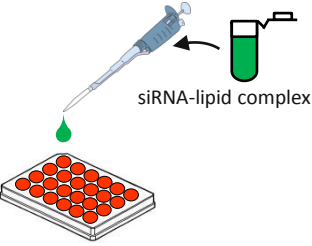
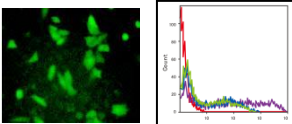


1-Step method (Reverse transfection method)

Timeline		Steps	Procedure Details																									
Day 0	1 <p>Reagent Dilution Buffer</p> <p>siRNA Dilution Buffer</p>	Dilute ScreenFect™ A plus Reagent* ¹ in Dilution Buffer, and then mix well ※1 Vortex the reagent before use Dilute siRNA in Dilution Buffer, and then mix well	<table border="1"> <thead> <tr> <th>Component</th> <th>96-well</th> <th>24-well</th> <th>12-well</th> <th>6-well</th> </tr> </thead> <tbody> <tr> <td>Dilution Buffer for ScreenFect™ A plus</td> <td>5 μL</td> <td>25 μL</td> <td>50 μL</td> <td>125 μL</td> </tr> <tr> <td>ScreenFect™ A plus Transfection Reagent</td> <td>0.1 – 0.3 μL</td> <td>0.5 – 1.5 μL</td> <td>1.0 - 3.0 μL</td> <td>2.5 – 7.5 μL</td> </tr> <tr> <td>Dilution Buffer for ScreenFect™ A plus</td> <td>5 μL</td> <td>25 μL</td> <td>50 μL</td> <td>125 μL</td> </tr> <tr> <td>siRNA</td> <td>1 pmol siRNA</td> <td>5 pmol siRNA</td> <td>10 pmol siRNA</td> <td>25 pmol siRNA</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	ScreenFect™ A plus Transfection Reagent	0.1 – 0.3 μL	0.5 – 1.5 μL	1.0 - 3.0 μL	2.5 – 7.5 μL	Dilution Buffer for ScreenFect™ A plus	5 μL	25 μL	50 μL	125 μL	siRNA	1 pmol siRNA	5 pmol siRNA	10 pmol siRNA	25 pmol siRNA
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2 <p>siRNA-lipid complex</p>	Add diluted siRNA 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 siRNA</td> <td>5 μL</td> <td>25 μL</td> <td>50 μL</td> <td>125 μL</td> </tr> <tr> <td>Diluted ScreenFect™ A plus Transfection Reagent</td> <td>5 μL</td> <td>25 μL</td> <td>50 μL</td> <td>125 μL</td> </tr> </tbody> </table>	Diluted siRNA	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 <p>Cultured cells</p>	Prepare required cells for transfection	<table border="1"> <tbody> <tr> <td>Adherent cells or suspension cells</td> <td>1.0-4.0 × 10⁴</td> <td>0.5-2.0 × 10⁵</td> <td>1.0-4.0 × 10⁵</td> <td>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 <p>Cell suspension</p>	Detach cells and prepare the cell suspension, and then transfer the required numbers of cell suspension to cell culture plate	Cell Detachment (Trypsin or Accutase®)																										
5 <p>siRNA-lipid complex</p>	Add siRNA-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>siRNA-lipid complex</td> <td>10 μL</td> <td>50 μL</td> <td>100 μL</td> <td>250 μL</td> </tr> <tr> <td>siRNA amount</td> <td>1 pmol siRNA</td> <td>5 pmol siRNA</td> <td>10 pmol siRNA</td> <td>25 pmol siRNA</td> </tr> <tr> <td>ScreenFect™ A plus Transfection Reagent used</td> <td>0.1 – 0.3 μL</td> <td>0.5 – 1.5 μL</td> <td>1.0 – 3.0 μL</td> <td>2.5 – 7.5 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	siRNA-lipid complex	10 μL	50 μL	100 μL	250 μL	siRNA amount	1 pmol siRNA	5 pmol siRNA	10 pmol siRNA	25 pmol siRNA	ScreenFect™ A plus Transfection Reagent used	0.1 – 0.3 μL	0.5 – 1.5 μL	1.0 – 3.0 μL	2.5 – 7.5 μL	Medium volume	100 μL	500 μL	1000 μL	2000 μL	
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Medium volume	100 μL	500 μL	1000 μL	2000 μL																								
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	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	
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 siRNA Dilution Buffer	Dilute ScreenFect™A plus Reagent ^{※1} in Dilution Buffer, and then mix well ^{※1} Vortex the reagent before use	Dilution Buffer for ScreenFect™A plus	5 μL	25 μL	50 μL	125 μL
			Dilute siRNA in Dilution Buffer, and then mix well	ScreenFect™A plus Transfection Reagent	0.1 μL	0.5 μL	1.0 μL	2.5 μL
3	Day 1	 siRNA-lipid complex	Add diluted siRNA 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	Dilution Buffer for ScreenFect™A plus	5 μL	25 μL	50 μL	125 μL
				siRNA	1 pmol siRNA	5 pmol siRNA	10 pmol siRNA	25 pmol siRNA
4		 siRNA-lipid complex	Add siRNA-lipid complex from step 3 to well of cell culture plate from step 1	Diluted siRNA	5 μL	25 μL	50 μL	125 μL
				Diluted ScreenFect™A plus Transfection Reagent	5 μL	25 μL	50 μL	125 μL
Final composition [per well]			96-well	24-well	12-well	6-well		
siRNA-lipid complex			10 μL	50 μL	100 μL	250 μL		
siRNA amount			1 pmol siRNA	5 pmol siRNA	10 pmol siRNA	25 pmol siRNA		
ScreenFect™A plus Transfection Reagent used			0.1 μL	0.5 μL	1.0 μL	2.5 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.				