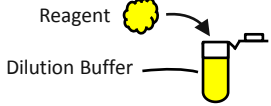
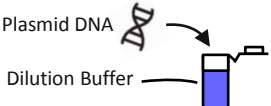
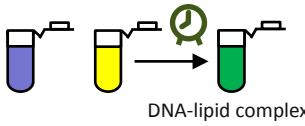

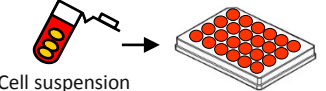
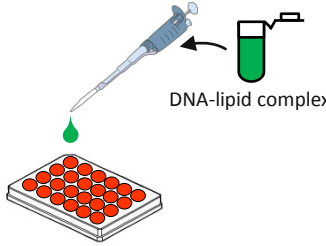
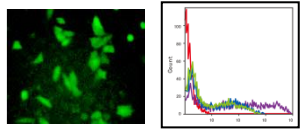


ScreenFect™A plus Transfection Protocol

The optimal condition for successful transfection varies. Start any new transfection by testing the recommended two concentrations of ScreenFect™A plus Reagent to determine an optimum amount.

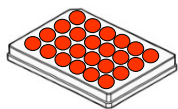
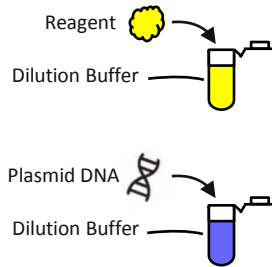

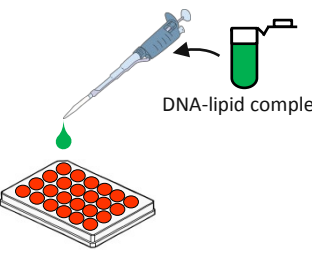
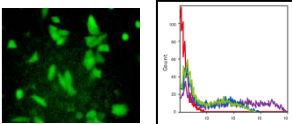
1-Step method (Reverse transfection method)

Timeline		Steps	Procedure Details										
Day 0	<p>1</p>  <p>Reagent Dilution Buffer</p>	<p>Dilute ScreenFect™A plus Reagent*¹ in Dilution Buffer, and then mix well *¹ Vortex the reagent before use</p>	Component		96-well		24-well		12-well		6-well		
	<p>2</p>  <p>Plasmid DNA Dilution Buffer</p>	<p>Dilute DNA in Dilution Buffer, and then mix well</p>	Dilution Buffer for ScreenFect™A plus		5 μL		25 μL		50 μL		125 μL		
	 <p>DNA-lipid complex</p>	<p>Add diluted DNA 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</p>	DNA : Transfection Reagent ratio		1 : 3	1 : 4	1 : 3	1 : 4	1 : 3	1 : 4	1 : 3	1 : 4	
	<p>3</p>  <p>Cultured cells</p>	<p>Prepare required cells for transfection</p>	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	
	<p>4</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>	Dilution Buffer for ScreenFect™A plus		5 μL		25 μL		50 μL		125 μL		
	<p>5</p>  <p>DNA-lipid complex</p>	<p>Add DNA-lipid complex from step 2 to well of cell culture plate from step 4</p>	DNA (0.1-2.5 μg / μL)		50 ng		250 ng		500 ng		1250 ng		
Day 1 ~	<p>6</p> 	<p>Visualize/analyze transfected cells</p>	Diluted DNA		5 μL		25 μL		50 μL		125 μL		
	<p>Cell Detachment (Trypsin or Accutase®)</p>		Diluted ScreenFect™A plus Transfection Reagent		5 μL		25 μL		50 μL		125 μL		
	<p>Final composition [per well]</p>		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		
				DNA-lipid complex		10 μL		50 μL		100 μL		250 μL	
				DNA 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	
				<p>Incubate cells for 1 day ~ at 37°C. Then, analyze transfected cells.</p>									
				<p>For support, please visit the http://screenfect.jp</p>									

ScreenFect™A plus Transfection Protocol

The optimal condition for successful transfection varies. Start any new transfection by testing the recommended two concentrations of ScreenFect™A plus Reagent to determine an optimum amount.

2-Step method (Forward transfection method)

Timeline		Steps	Procedure Details																																																							
1	Day 0	 <p>Pre-Cultured cells</p>	<p>Seed cells to be 70-90% confluent at transfection</p>	<p>Seed cells to be 70-90% confluent at transfection. The medium replacement may improve a transfection efficiency in a 2-Step method.</p>																																																						
				<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		 <p>Reagent Dilution Buffer</p> <p>Plasmid DNA Dilution Buffer</p>	<p>Dilute ScreenFect™A plus Reagent^{※1} in Dilution Buffer, and then mix well ^{※1} Vortex the reagent before use</p> <p>Dilute DNA in Dilution Buffer, and then mix well</p>	<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>DNA : 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		DNA : 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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