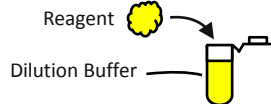
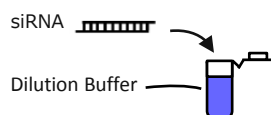
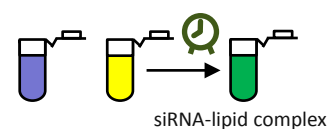
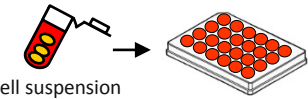
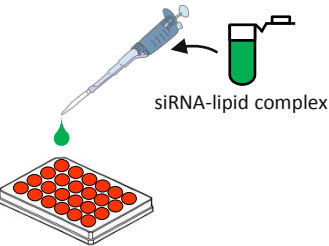
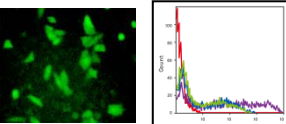


ScreenFect™ A Transfection Protocol

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

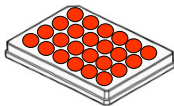
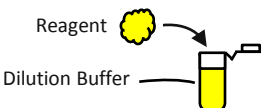
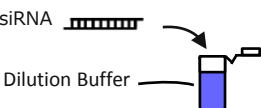
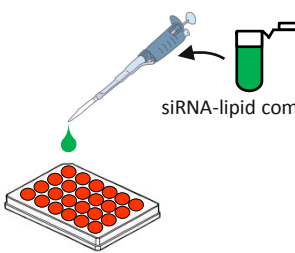
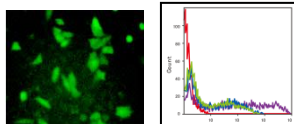
1-Step method (Reverse transfection method)

Timeline		Steps	Procedure Details					
Day 0	1	 Reagent Dilution Buffer	Dilute ScreenFect™A Reagent※ ¹ in Dilution Buffer, and then mix well ※ ¹ Vortex the reagent before use	Component	96-well	24-well	12-well	6-well
		 siRNA Dilution Buffer	Dilute siRNA in Dilution Buffer, and then mix well	Dilution Buffer for ScreenFect™A	5 μL	25 μL	50 μL	125 μL
	2	 siRNA-lipid complex	Add diluted siRNA to diluted ScreenFect™A Reagent, and then incubate for 5 minutes ~ at room temperature※ ² ※ ² Incubation is available until the step 4 has been completed	ScreenFect™A Transfection Reagent	0.1-0.3 μL	0.5-1.5 μL	1.0-3.0 μL	3.5-7.5 μL
	3	 Cultured cells	Prepare required cells for transfection	Dilution Buffer for ScreenFect™A	5 μL	25 μL	50 μL	125 μL
	4	 Cell suspension	Detach cells and prepare the cell suspension, and then transfer the required numbers of cell suspension to cell culture plate	siRNA	2-3 pmol	10-20 pmol	20-40 pmol	20-60 pmol
	5	 siRNA-lipid complex	Add siRNA-lipid complex from step 2 to well of cell culture plate from step 4	Diluted siRNA	5 μL	25 μL	50 μL	125 μL
				Diluted ScreenFect™A Transfection Reagent	5 μL	25 μL	50 μL	125 μL
				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
				Cell Detachment (Trypsin or Accutase®)				
Day 1 ~	6		Visualize/analyze transfected cells	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	2-3 pmol	10-20 pmol	20-40 pmol	20-60 pmol
				ScreenFect™A Transfection Reagent used	0.1-0.3 μL	0.5-1.5 μL	1.0-3.0 μL	3.5-7.5 μL
				Medium volume	100 μL	500 μL	1000 μL	2000 μL
				Incubate cells for 1 day ~ at 37°C. Then, analyze transfected cells.				
				For support, please visit the http://screenfect.jp				

ScreenFect™ A Transfection Protocol

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

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	Dilute ScreenFect™A Reagent※ ¹ in Dilution Buffer, and then mix well ※ ¹ Vortex the reagent before use	Dilution Buffer for ScreenFect™A	5 μL	25 μL	50 μL	125 μL
				 siRNA Dilution Buffer	ScreenFect™A Transfection Reagent	0.1-0.3 μL	0.5-1.5 μL	1.0-3.0 μL
3	Day 1	 siRNA-lipid complex	Add diluted siRNA to diluted ScreenFect™A 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	5 μL	25 μL	50 μL
				siRNA	2-3 pmol	10-20 pmol	20-40 pmol	20-60 pmol
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 Transfection Reagent	5 μL	25 μL	50 μL	125 μL
5	Day 2 ~		Visualize/analyze transfected cells	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	2-3 pmol	10-20 pmol	20-40 pmol	20-60 pmol
				ScreenFect™A Transfection Reagent used	0.1-0.3 μL	0.5-1.5 μL	1.0-3.0 μL	3.5-7.5 μL
				Medium volume	100 μL	500 μL	1000 μL	2000 μL
				Incubate cells for 1 day ~ at 37°C. Then, analyze transfected cells.				
				For support, please visit the http://screenfect.jp				