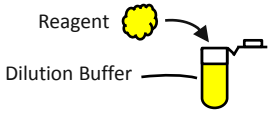
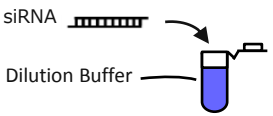
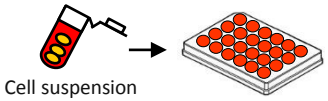
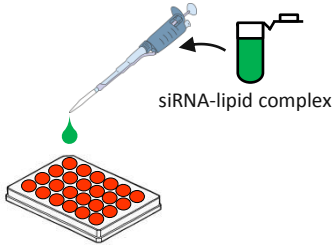
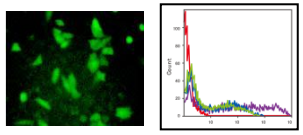
ScreenFect™siRNA Quick Protocol

ScreenFect™siRNA Transfection Protocol

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

1-Step method (Reverse transfection method)

Timeline	Steps
1 	**Dilute ScreenFect™siRNA Reagent※1 in Dilution Buffer, and then mix well** **※1 Vortex the reagent before use**
1 	**Dilute siRNA in Dilution Buffer, and then mix well**
2 	**Add diluted siRNA to diluted ScreenFect™siRNA Reagent, and then incubate for 5 minutes ～ at room temperature※2** **※2 Incubation is available until the step 4 has been completed**
Day 0 3 	**Prepare required cells for transfection**
4 	**Detach cells and prepare the cell suspension, and then transfer the required numbers of cell suspension to cell culture plate**
5 	**Add siRNA-lipid complex from step 2 to well of cell culture plate from step 4**
Day 1～ 6 	**Visualize/analyze transfected cells**

Procedure Details

Component	96-well	24-well	12-well	6-well
Dilution Buffer for ScreenFect™siRNA	5 µL	25 µL	50 µL	125 µL
ScreenFect™siRNA Transfection Reagent	0.1 µL	0.5 µL	1.0 µL	2.5µL
Dilution Buffer for ScreenFect™siRNA	5 µL	25 µL	50 µL	125 µL
siRNA	1 pmol siRNA	5 pmol siRNA	10 pmol siRNA	25 pmol siRNA
Diluted siRNA	5 µL	25 µL	50 µL	125 µL
Diluted ScreenFect™siRNA 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*®)				

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™siRNA 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

Incubate cells for 1 day ～ at 37°C. Then, analyze transfected cells.

For support, please visit the http://screenfect.jp

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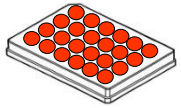
2-Step method (Forward transfection method)

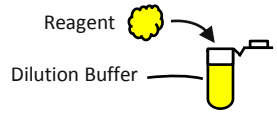
Timeline

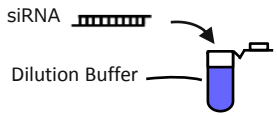
1 Day 0



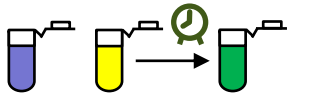
Pre-Cultured cells

2



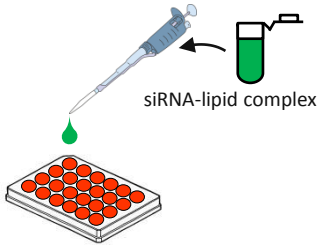


3 Day 1

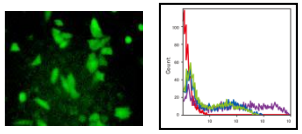


siRNA-lipid complex

4



5 Day 2～



Steps

Seed cells to be 70-90% confluent at transfection

Dilute ScreenFect™siRNA Reagent※1 in Dilution Buffer, and then mix well
※1 Vortex the reagent before use

Dilute siRNA in Dilution Buffer, and then mix well

Add diluted siRNA to diluted ScreenFect™siRNA Reagent, and then incubate for 5 minutes ～ at room temperature※2
※2 Incubation is available until the step 4 has been completed

Add siRNA-lipid complex from step 3 to well of cell culture plate from step 1

Visualize/analyze transfected cells

Procedure Details

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.				
Dilution Buffer for ScreenFect™siRNA	5 µL	25 µL	50 µL	125 µL
ScreenFect™siRNA Transfection Reagent	0.1 µL	0.5 µL	1.0 µL	2.5 µL
Dilution Buffer for ScreenFect™siRNA	5 µL	25 µL	50 µL	125 µL
siRNA	1 pmol siRNA	5 pmol siRNA	10 pmol siRNA	25 pmol siRNA
Diluted siRNA	5 µL	25 µL	50 µL	125 µL
Diluted ScreenFect™siRNA 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™siRNA 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
Incubate cells for 1 day ～ at 37℃. Then, analyze transfected cells.				

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