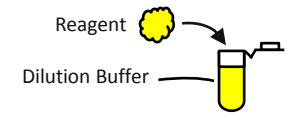
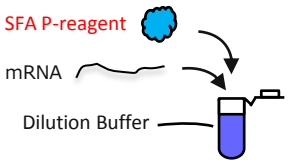
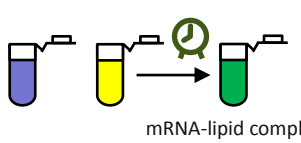
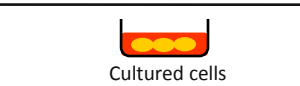
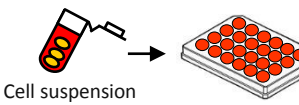
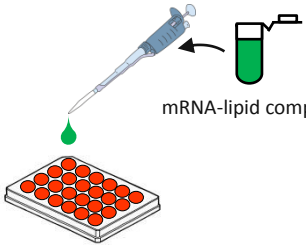
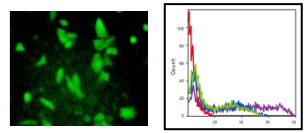
ScreenFect™mRNA (+SFA P-reagent) Quick Protocol

ScreenFect™mRNA Transfection Protocol

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

1-Step method (Reverse transfection method)

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

Component	96-well		24-well		12-well		6-well	
Dilution Buffer for ScreenFect™mRNA	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™mRNA 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
Dilution Buffer for ScreenFect™mRNA	5 µL		25 µL		50 µL		125 µL	
mRNA (0.1-2.5 µg / µL)	50 ng		250 ng		500 ng		1250 ng	
SFA P-reagent (2µL / µg mRNA)	0.1 µL		0.5 µL		1.0 µL		2.5 µL	
Diluted mRNA (+SFA P-reagent)	5 µL		25 µL		50 µL		125 µL	
Diluted ScreenFect™mRNA 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
mRNA-lipid complex	10 µL	50 µL	100 µL	250 µL
mRNA amount	50 ng	250 ng	500 ng	1250 ng
SFA P-reagent used	0.1 µL	0.5 µL	1.0 µL	2.5 µL
ScreenFect™mRNA 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

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

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

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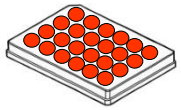
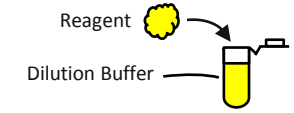
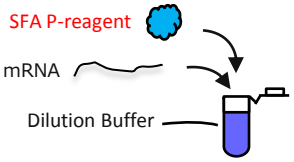
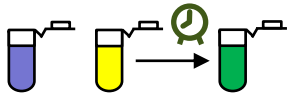
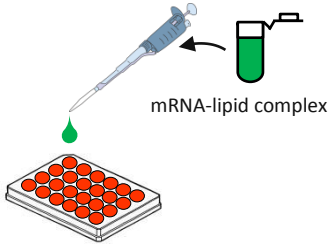
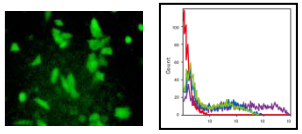
ScreenFect™mRNA Transfection Protocol

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
1 Day 0  Pre-Cultured cells	**Seed cells to be 70-90% confluent at transfection**
2 Day 1 	**Dilute ScreenFect™mRNA Reagent※1 in Dilution Buffer, and then mix well** **※1 Vortex the reagent before use**
	**Dilute mRNA and SFA P-reagent in Dilution Buffer, and then mix well**
3 Day 1  mRNA-lipid complex	**Add diluted mRNA (+SFA P-reagent) to diluted ScreenFect™mRNA Reagent, and then incubate for 5 minutes ～ at room temperature※2** **※2 Incubation is available until the step 4 has been completed**
4 	**Add mRNA-lipid complex from step 3 to well of cell culture plate from step 1**
5 Day 2～ 	**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™mRNA	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™mRNA 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
Dilution Buffer for ScreenFect™mRNA	5 µL	25 µL	50 µL	125 µL
mRNA (0.1-2.5 µg / µL)	50 ng	250 ng	500 ng	1250 ng
SFA P-reagent (2µL / µg mRNA)	0.1 µL	0.5 µL	1.0 µL	2.5 µL
Diluted mRNA (+SFA P-reagent)	5 µL	25 µL	50 µL	125 µL
Diluted ScreenFect™mRNA Transfection Reagent	5 µL	25 µL	50 µL	125 µL
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
SFA P-reagent used	0.1 µL	0.5 µL	1.0 µL	2.5 µL
ScreenFect™mRNA 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

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

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