PromoFectin-siRNA
Cell Transfection Reagent

PromoKine

Instruction Manual

Cat.No. PK-CT-2000-RNA-050
PK-CT-2000-RNA-100
PK-CT-2000-RNA-500

Contents

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0.5 ml (PK-CT-2000-RNA-050), 1 ml (PK-CT-2000-RNA-100), and 5 x 1 ml (PK-CT-2000-RNA-500) PromoFectin-siRNA Transfection Reagent. One ml of Promofectin siRNA transfection reagent is sufficient to perform ca. 500 to 1000 transfections (using 1 nM of siRNA) in 24-well plates.

Product Description

PromoFectin-siRNA is a powerful transfection reagent that ensures reproducible transfection and efficient gene silencing in mammalian cells using siRNA/miRNA oligonucleotides. PromoFectin-siRNA provides more than 90% silencing efficiency at nanomolar siRNA concentration in a wide variety of cells; hence avoiding off-target effects.

Materials and Methods

1 Standard siRNA Transfection Protocol for Adherent Cells

1.1 Cell Seeding

For optimal transfection of standard adherent cells using PromoFectin-siRNA, cells should be seeded the day before transfection to reach 30-50% confluency at the time of transfection (refer to Table 1 for recommended number of cells to be seeded according to the culture vessel formats).

Table 1. Recommended number of cells to seed the day before transfection.

Culture vessel	Number of adherent cells to seed	Surface area per well (cm^2)	Volume of medium per well to seed the cells (ml)
96-well	5 000 ± 2 500	0.3	0.2
24-well	25 000 ± 10 000	1.9	1
12-well	50 000 ± 20 000	3.8	2
6-well / 3.5 cm	150 000 ± 50 000	9.4	4
6 cm / flask 25 cm^2	400 000 ± 100 000	25 - 28	8
10 cm / flask 75 cm^2	1 x 10^6 ± 250 000	75 - 78.5	15
14 cm / flask 175 cm^2	2 x 10^6 - 5 x 10^6	153 - 175	20

1.2 Transfection of Adherent Cells

As starting conditions for your gene silencing experiment, we recommend testing siRNA concentrations ranging from 1 nM to 10 nM, as the optimal siRNA concentration depends largely on the target gene, the cell type, the siRNA potency, the half-life of the target mRNA and the turnover of the target protein. Please note that off-target effects are usually minimized at lower siRNA concentrations. The volume of PromoFectin-siRNA should be adjusted according to the siRNA concentration and the plate size as shown in Table 2. The transfection conditions are detailed in Table 3 for all culture plate formats.

Note:

- Check the concentration of the siRNA duplexes, even if provided by the manufacturer.
- Use RNase free and apyrogenic materials such as tips, tubes, buffers.

1.2.1 siRNA Transfection Protocol using 1 nM siRNA

The following protocol is given for transfection of siRNA duplexes at 1 nM per well in a 24-well plate, refer to Table 2 for transfection in other culture formats.

1. For each well, dilute 0.6 pmoles (8.4 ng) of siRNA duplexes into 100 µl of medium without serum or in Opti-MEM®. Mix by pipetting up and down.
2. Add 2 µl of PromoFectin-siRNA to the 100 µl of siRNA duplexes.
3. Immediately homogenize by vortexing for 10 seconds.
4. Incubate for 10 minutes at room temperature to allow transfection complexes to form between siRNA duplexes and PromoFectin-siRNA. Do not exceed 30 minutes.
5. During complex formation, remove the growth medium and add 0.5 ml of fresh pre-warmed complete medium per well.
6. Add 100 µl of transfection mix onto the cells and homogenize by gently swirling the plate. The final volume is 600 µl and the siRNA concentration is 1 nM.

7. Incubate the plate at 37°C.
8. Gene silencing is usually measured between 24 to 72 hours for mRNA levels and 48 to 96 hours for proteins.

Table 2. Recommended transfection conditions in various cell culture formats at 1 nM siRNA.

Culture vessel	siRNA duplexes (pmoles)	Amount of siRNA per well	Volume of PromoFectin-siRNA	Volume of medium w/o serum for complexation	Volume of complete medium on cells	Final volume
96-well	0.17	2.4 ng	0.75 ± 0.5 µl	50 µl	125 µl	175 µl
24-well	0.6	8.4 ng	2 ± 1 µl	100 µl	500 µl	600 µl
12-well	1.2	17 ng	4 ± 2 µl	200 µl	1 ml	1.2 ml
6-well / 3.5 cm	2.2	31 ng	8 ± 4 µl	200 µl	2 ml	2.2 ml
6 cm / flask 25 cm^2	4.4	62 ng	15 ± 5 µl	400 µl	4 ml	4.4 ml
10 cm / flask 75 cm^2	10.5	147 ng	40 ± 10 µl	500 µl	10 ml	10.5 ml

1.2.2 Transfection Conditions using 10 to 50 nM siRNA

When working at siRNA concentrations ranging from 10 to 50 nM, use recommended conditions indicated in Table 3.

Table 3. Recommended conditions to transfect adherent cells in different cell culture vessels from 10 to 50 nM siRNA.

Culture vessel	Volume of PromoFectin-siRNA	Volume of medium w/o serum for complexe formation	Volume of complete medium on cells	Final volume
96-well	1 ± 0.5 µl	50 µl	125 µl	175 µl
24-well	3 ± 1 µl	100 µl	500 µl	600 µl
12-well	6 ± 2 µl	200 µl	1 ml	1.2 ml
6-well / 3.5 cm	12 ± 4 µl	200 µl	2 ml	2.2 ml
6 cm / flask 25 cm^2	20 ± 5 µl	400 µl	4 ml	4.4 ml

2 siRNA Transfection of Suspension Cells

2.1 Cell Seeding

For optimal transfection conditions of suspension cells with PromoFectin-siRNA, cells should be seeded the day of transfection in a reduced volume compared to usual culture conditions. Refer to Table 4 for the recommended number of cells to seed according to the culture vessel formats and for the advised volume of complete medium.

Table 4. Recommended number of suspension cells to seed the day of transfection.

Culture vessel	Number of suspension cells to seed the day of transfection	Volume of medium per well
384-well	5 000 - 10 000	25 µl
96-well	10 000 - 20 000	50 µl
24-well	100 000 - 200 000	200 µl
12-well	200 000 - 400 000	500 µl
6-well / 3.5 cm	500 000 - 2 x 10^6	1 ml
6 cm / flask 25 cm^2	2 x 10^6 - 5 x 10^6	2 ml

2.2 siRNA Transfection of Suspension Cells

In order to optimize endogenous gene silencing, we recommend testing a range of siRNA concentrations from 5 nM to 20 nM. The volume of PromoFectin-siRNA needs to be adjusted accordingly, depending on the siRNA concentration as described in Table 5.

For detailed transfection conditions at 5 nM siRNA, please refer to Table 6.

Table 5. Recommended volumes of PromoFectin-siRNA according to the siRNA concentration and the plate format for transfection of cells grown in suspension.

Final siRNA concentration	Plate format	Volume of PromoFectin-siRNA reagent/well
1 to 20 nM	384-well	1 ± 0.5 µl
	96-well	2 ± 1 µl
	24-well	3 ± 2 µl
	6-well or 35 mm	10 ± 8 µl
20 to 50 nM	384-well	1.5 ± 0.5 µl
	96-well	3 ± 1 µl
	24-well	5 ± 2 µl
	6-well or 35 mm	15 ± 8 µl

Preparation of the complexes and transfection procedure

The following protocol is given for transfection of siRNA duplexes at 5 nM per well in a 24-well plate. See Table 6 for transfection in other culture formats.

1. For each well, dilute 1.5 pmoles (21 ng) of siRNA duplexes into 100 µl medium without serum or in Opti-MEM®. Mix by pipetting up and down.
2. Add 4 µl of PromoFectin-siRNA to the 100 µl siRNA duplexes solution.
3. Mix immediately for 10 seconds (vortex).
4. Incubate for 15 minutes at room temperature to allow PromoFectin-siRNA/siRNA complexes to form (do not exceed 30 minutes).
5. Add the 100 µl PromoFectin-siRNA®/siRNA mix per well into 0.2 ml of cells suspension in growth medium, and homogenize by gently swirling the plate. The final volume is 300 µl and the siRNA concentration is 5 nM.
6. Incubate the plate at 37°C.
7. After 4 to 6 hours, add 0.7 ml of complete medium and incubate as before.

8. Gene silencing is usually measured between 24 to 72 hours for mRNA levels and 48 to 96 hours for proteins.

Table 6. Recommended conditions for siRNA transfection at 5 nM in suspension cells.

Culture vessel	Volume of cell suspension	siRNA duplexes (pmoles)	Amount of siRNA per well	Volume of PromoFectin-siRNA	Volume of medium w/o serum for complexation	Volume of medium to add after 4 - 6 h
384-well	25 µl	0.25	3.75 ng	1 ± 0.5 µl	25 µl	0 µl
96-well	50 µl	0.5	7.5 ng	2 ± 1 µl	50 µl	100 µl
24-well	200 µl	1.5	21 ng	3 ± 2 µl	100 µl	0.7 ml
12-well	500 µl	3.5	49 ng	6 ± 4 µl	200 µl	1 ml
6-well / 3.5 cm	1 ml	6	84 ng	10 ± 8 µl	200 µl	2 ml
6 cm / flask 25 cm^2	2 ml	12	168 ng	15 ± 10 µl	400 µl	4 ml

Note: *For other siRNA concentrations, please adjust the conditions accordingly.*

3 miRNA Transfection

PromoFectin-siRNA is suitable for transfection of miRNA and miRNA-related molecules by using the standard protocol, described in Section 1.2. for adherent and Section 2.2. for suspension cells.

Troubleshooting

Observations	Actions
Low silencing efficiency	Increase the siRNA concentration per well.
	Increase the volume of PromoFectin-siRNA per well.
	Check silencing efficiency at various time points after transfection from 24 to 96 h.
	Use Opti-MEM® to dilute the siRNA.
	Ensure that adherent cells are 30-50% confluent the day of transfection. For small cells and slow growing cell types, seed approximately 2 times more cells per well to reach the adequate confluence.
	Check all reagents are RNase free.
	Ensure that your siRNAs are high-quality (PAGE purified and desalted).
	Check siRNA duplexes concentration and annealing.
	Decrease the volume during transfection by half and gently centrifuge the plate (5 min at 180 g). After 4 hours, add 0.5 ml of medium.
Cellular toxicity	Reduce the incubation time of Promofectin siRNA/siRNA complexes with the cells by changing medium 4 to 6 h after transfection or simply adding medium to the well.
	Decrease the volume of Promofectin siRNA used in the transfection assay.
	Check that silencing the target gene does not affect cell viability.

Shipping & Storage

PromoFectin-siRNA should be stored tightly capped at 4°C upon arrival. Do not freeze.

PromoFectin-siRNA, as guaranteed by the Certificate of Analysis, will be stable for at least one year when stored appropriately.

Quality Control

Every batch of PromoFectin-siRNA is tested in house in a transfection assay on A549-Luc cells, constitutively expressing the Luciferase gene. The silencing efficiency obtained using 1 nM siRNA and PromoFectin-siRNA for each batch is indicated on the Certificate of Analysis.

Ordering Information

Product Name	Size	Catalog Number
PromoFectin-siRNA	0.5 ml	PK-CT-2000-RNA-050
PromoFectin-siRNA	1 ml	PK-CT-2000-RNA-100
PromoFectin-siRNA	5 x 1 ml	PK-CT-2000-RNA-500

For in vitro research use only.
Not for diagnostic or therapeutic procedures.

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