PromoFectin Transfection Reagent

PromoKine

Instruction Manual

Product Name	Product Description	Size	Catalog Number
PromoFectin	PromoFectin Transfection Reagent	0.1 ml 0.5 ml 1.0 ml	PK-CT-2000-10 PK-CT-2000-50 PK-CT-2000-100

Content

1 ml of PromoFectin is sufficient to perform up to 500 transfections in 24-well plates.

Formulation and Storage

PromoFectin is provided as a sterile aqueous solution and shipped at room temperature. Upon arrival it should be store at 4°C. PromoFectin is stable for 1 year at 4°C.

Transient transfection of adherent cells

Preparation of Cells

For optimal transfection conditions with PromoFectin, the cells should be 50-60% confluent. Typically, for transfection in 24-well plates, 50 000 to 100 000 cells are seeded per well, 24 hours before transfection. For other culture formats, see Table 1.

Table 1: Number of cells to seed the day before transfection

Culture vessel	Number of adherent cells to seed	Volume of medium per well or plate
96-well	10 000 - 17 000	0.2 ml
48-well	25 000 - 50 000	0.5 ml
24-well	50 000 - 100 000	1 ml
12-well	80 000 - 200 000	2 ml
6-well/ 35 mm	200 000 - 400 000	4 ml
6 cm/flask 25 cm^2	400 000 – 800 000	10 ml
10 cm/flask 75 cm^2	1 000 000 – 2 000 000	20 ml
14 cm/flask 153 cm^2	2 x 10^6 – 5 x 10^6	40 ml

Transfection procedure

The following protocol is given for transfection in 24-well plates.

Use 2 µl of PromoFectin and 1 µg of DNA per well as follows. See table 2 for other culture vessels.

1. For each well, dilute 1 µg of DNA into 50 µl of culture medium without serum or Opti-MEM®. Vortex gently and spin down briefly.
2. For each well, dilute 2 µl of PromoFectin solution into 50 µl of culture medium without serum or Opti-MEM®. Vortex gently and spin down briefly.
3. Add the 50 µl PromoFectin solution to the 50 µl DNA solution all at once
4. Vortex-mix the solution immediately and spin down briefly.
5. Incubate for 15 to 30 minutes at room temperature.
6. Add the 100 µl PromoFectin /DNA mix drop-wise onto the serum containing medium in each well and homogenize the mixture by gently swirling the plate.
7. After 24 to 48 hours assess reporter gene activity*.

Table 2: Transfection mix preparation for different cell culture formats

Culture vessel	Amount of DNA (µg)	Volume of PromoFectin (µl)	Volume to dilute DNA and PromoFectin (µl)	Total volume of transfection mix per well (µl)
96-well	0.25	0.5	10	20
48-well	0.5	1	25	50
24-well	1	2	50	100
12-well	2	4	50	100
6-well	3	6	100	200
6 cm	5	10	250	500
10 cm	7 - 8	14 - 16	500	1000
14 cm	10 - 12	20 - 24	1000	2000

Transfection efficiency

PromoFectin is not affected by the presence of serum during transfection. Therefore, the transfection mix can be added directly to the serum containing medium. Usually, transfection efficiencies can be improved by using smaller volumes of medium or/and by centrifugation of the culture plate (5 min at 280g at room temperature). If cytotoxicity is observed, the transfection mix can be removed after a 2-4 hours incubation period. Replace by fresh complete culture medium.

Note: For siRNA transfection, we recommend to use 2 µl PromoFectin and 1 µg siRNA per well in a 24-well plate. For achieving optimal results, it might be necessary to vary the amounts and ratio of transfection reagent and siRNA to a certain extent.

Transient transfection of suspension cells

Preparation of Cells

Seed the following number of cells according to the culture vessel format.

* **Note:** Reporter gene vectors (e.g. Luciferase, beta-Galactosidase, GFP) as well as the respective cell-based reporter assays for Luciferase, beta-Galactosidase and GFP are also available at PromoKine (see www.promokine.info).

Table 3: Number of cells to seed

Culture vessel	Number of cells	Amount of DNA	Volume of PromoFectin (µl)	Final volume of transfection mix (µl/ well)
96-well	$2 .10^4$ - $5 .10^4$	0.2 - 0.4 µg	0.4 – 0.8 µl	20
48-well	$5 .10^4$ – 10^5	0.5 - 1 µg	1 – 2 µl	50
24-well	10^5 – $2 . 10^5$	0.5 - 1 µg	1 – 2 µl	100
12-well	$2 . 10^5$ – $5 . 10^5$	1 - 2 µg	2 – 4 µl	100
6-well / 35 mm	$5 . 10^5$ – $2 . 10^6$	2 - 4 µg	4 – 8 µl	200
6 cm / flask 25 cm^2	$2 . 10^6$ – $5 . 10^6$	4 – 8 µg	8 – 16 µl	250
10 cm / flask 75 cm^2	$5 . 10^6$ - 10^7	8 – 16 µg	16 – 32 µl	500

Transfection procedure

The following protocol is given for transfection in 6-well plates.

1. For each well, dilute 2 – 4 µg of DNA into 100 µl of culture medium without serum or Opti-MEM®. Vortex gently and spin down briefly.
2. For each well, dilute 4 – 8 µl of PromoFectin solution (see table 3) into 100 µl of culture medium without serum or Opti-MEM®. Vortex gently and spin down briefly.
3. Add the 100 µl PromoFectin solution to the 100 µl DNA solution all at once.
4. Vortex-mix the solution immediately and spin down briefly.
5. Incubate for 15 to 30 minutes at room temperature.
6. Add the 200 µl transfection mix drop-wise onto cells in serum containing medium (8. 10^5 cells per well). Homogenize.
7. Incubate at 37°C and 5% CO_2 in a humidified atmosphere.
8. Transfection experiments are usually stopped after 24 to 48 hours and gene activity assessed. Cells growing in suspension are collected by centrifugation at 400g and then resuspended in the desired medium or buffer.

The following protocol is given for transfection in 24-well plates.

1. For each well, dilute 0.5 – 1 µg of DNA into 50 µl of of culture medium without serum or Opti-MEM®. Vortex gently and spin down briefly.
2. For each well, dilute 1 – 2 µl of PromoFectin solution (see table 3) into 50 µl of of culture medium without serum or Opti-MEM®. Vortex gently and spin down briefly.
3. Add the 50 µl PromoFectin solution to the 50 µl DNA solution all at once.
4. Vortex-mix the solution immediately and spin down briefly.
5. Incubate for 15 to 30 minutes at room temperature.
6. Add the 100 µl transfection mix drop-wise onto the cells in serum containing medium (2 x 10^5 cells per well) and homogenize gently.
7. Incubate at 37°C and 5% CO_2 in a humidified atmosphere.
8. After 24 to 48 hours assess reporter gene activity.

Cells growing in suspension are collected by centrifugation at 400g and then resuspended in the desired medium or buffer.

Stable transfection

For stable transfection, perform transfection in 6-well plates or 60 mm plates according to the protocol described in section A. Start selection with appropriate antibiotic 24 – 48 h after transfection

Troubleshooting

Low transfection efficiency

- Optimize the PromoFectin/DNA ratio starting from 1 µl Promofectin/µg DNA up to 4µl PromoFectin/µg DNA.
- Optimize the amount of plasmid DNA used in the transfection assay.
- Use high-quality plasmid preparation, free of RNA (the $OD_{260/280}$ ratio should be greater than 1.8).
- Decrease the culture medium volume.
- Ensure that adherent cells are 50-60% confluent the day of transfection.
- Perform a positive control transfection experiment with a well-characterized reporter gene (beta-Gal or Luciferase from commercially available plasmids, e.g. PromoKine's reporter vectors pPK-CMV-R1 [PK-MB-P010200] or pPK-CMV-R3 [PK-MB-P030200]; the respective beta-Gal and Luciferase reporter assays are also available at PromoKine).

Cellular toxicity

- Decrease the amount of plasmid DNA used in the transfection assay
- Check DNA concentration and ensure that you use no more than 2 µl of PromoFectin for 1 µg of DNA.
- Reduce the incubation time of the transfection mix with the cells.
- Verify the toxicity of the expressed protein. If the expressed protein is toxic for the cells, reduce the amount of plasmid DNA used in the transfection assay.
- Make sure that the plasmid preparation is endotoxin-free.

Intended Use

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

PromoCell GmbH

Sickingenstr. 63/65
69126 Heidelberg
Germany

Email: info@promokine.info
www.promokine.info

North America
Phone: 1 – 866 – 251 – 2860 (toll free)
Fax: 1 – 866 – 827 – 9219 (toll free)

Deutschland
Telefon: 0800 – 776 66 23 (gebührenfrei)
Fax: 0800 – 100 83 06 (gebührenfrei)

France
Téléphone: 0800 90 93 32 (ligne verte)
Téléfax: 0800 90 27 36 (ligne verte)

United Kingdom
Phone: 0800 – 96 03 33 (toll free)
Fax: 0800 – 169 85 54 (toll free)

Other Countries
Phone: +49 6221 – 649 34 0
Fax: +49 6221 – 649 34 40