PromoFectin-Macrophage Transfection Reagent

PromoKine

Instruction Manual

Product Name	Product Description	Size	Catalog Number
PromoFectin-Macrophage	PromoFectin-Macrophage Transfection Reagent	0,1 ml 0,5 ml	PK-CT-2000-MAC-10 PK-CT-2000-MAC-50

Content

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

Formulation and Storage

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

Preparation of Cells

Primary macrophages should be cultured in the presence of GM-CSF for 7 to 10 days prior to transfection. For establish cell lines such as RAW 264.7, maturation with GM-CSF is not required.
For optimal transfection conditions with PromoFectin-Macrophage, the cells should be 50-60% confluent. Typically, for transfection in 24-well plates, 100.000 cells are seeded per well, 24 hours before transfection (see table 1 for other culture plate format).

Table 1. Number of cells to seed before transfection

Culture vessel	Number of RAW 264.7 cells to seed one day before	Number of primary macrophages to seed one day before	Volume of medium per well
24-well	50 – 100 000	100 000	1 ml
12-well	100 - 200 000	200 000	2 ml
6-well/ 35 mm	200 - 400 000	400 000	4 ml

Transfection procedure for Primary Macrophages

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

Use 1 µl of PromoFectin-Macrophage and 0.5 µg of DNA per well as follows. See table 2 for other culture vessels.

1. For each well, dilute 0.5 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 1 µl of PromoFectin-Macrophage solution into 50 µl of culture medium without serum or Opti-MEM®. Vortex gently and spin down briefly.
3. Add the 50 µl PromoFectin-Macrophage 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-Macrophage /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

For Primary Macrophages

Culture vessel	Amount of DNA(µg)	Volume of PromoFectin-Macrophage (µl)	Volume to dilute DNA and PromoFectin-Macrophage (µl)	Total volume of transfection mix per well (µl)
24-well	0.5	1	50	100
12-well	1	2	50	100
6-well	1.5	3	100	200

Transfection procedure for RAW 264.7

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

Use 6.4 µl of PromoFectin-Macrophage and 2 µg of DNA per well as follows. See table 3 for other culture vessels.

1. For each well, dilute 2 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 6.4 µl of PromoFectin-Macrophage solution into 50 µl of culture medium without serum or Opti-MEM®. Vortex gently and spin down briefly.
3. Add the 50 µl PromoFectin-Macrophage 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-Macrophage /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 3. Transfection mix preparation for different cell culture formats

For RAW 264.7 establish cell line

Culture vessel	Amount of DNA(µg)	Volume of PromoFectin-Macrophage (µl)	Volume to dilute DNA and PromoFectin-Macrophage (µl)	Total volume of transfection mix per well (µl)
24-well	2	6.4	50	100
12-well	4	12.8	50	100
6-well	6	19.2	100	200

Transfection efficiency

PromoFectin-Macrophage 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.

Troubleshooting

Low transfection efficiency

- Optimize the PromoFectin-Macrophage/DNA ratio starting from 1 µl PromoFectin-Macrophage/µg DNA up to 4 µl PromoFectin-Macrophage/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 (GFP or β-Gal from commercially available plasmid such as pPK-CMV-R4 or pPK-CMV-R1; order nos. PK-MB-P040200 and PK-MB-P010200. Reporter gene 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 that 3.2 µl of PromoFectin-Macrophage 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.

Related Products

Order Number	Product Name	Size
PK-CT-2000-10	PromoFectin	0.1 ml
PK-CT-2000-50	PromoFectin	0.5 ml
PK-CT-2000-100	PromoFectin	1 ml
PK-CT-2000-HUV-10	PromoFectin-HUVEC	0.1 ml
PK-CT-2000-HUV-50	PromoFectin-HUVEC	0.5 ml
PK-CT-2000-HEP-10	PromoFectin-Hepatocyte	0.1 ml
PK-CT-2000-HEP-50	PromoFectin-Hepatocyte	0.5 ml
PK-CT-2000-InC-50	PromoFectin-Insect	0.5 ml
PK-CT-2000-InC-100	PromoFectin-Insect	1 ml
PK-CT-2000-RNA-50	PromoFectin-siRNA	0.2 ml (50 reactions)
PK-CT-2000-RNA-200	PromoFectin-siRNA	0.75 ml (200 reactions)
PK-CT-2000-RNA-1000	PromoFectin-siRNA	5 x 0.75 ml (1,000 reactions)
PK-CT-2000-POL-24	PromoFectin-Polypeptide	24 reactions
PK-CT-2000-POL-24Q	PromoFectin-Polypeptide	24 reactions (single-use tubes)
PK-CT-2000-POL-96	PromoFectin-Polypeptide	96 reactions
PK-CT-2000-POL-96Q	PromoFectin-Polypeptide	96 reactions (single-use tubes)
PK-CT-2000-NEU-015	PromoFectin-Neuron	0.15 ml (15-60 reactions)
PK-CT-2000-NEU-075	PromoFectin-Neuron	0.75 ml (75-300 reactions)
PK-CT-2000-NEU-375	PromoFectin-Neuron	5 x 0.75 ml
PK-CT-2000-CL-075	PromoFectin-CellLine	0.75 ml
PK-CT-2000-CL-150	PromoFectin-CellLine	1.5 ml
PK-CT-2000-OLI-050	PromoFectin-Oligo	0.5 ml
PK-CT-2000-OLI-100	PromoFectin-Oligo	1 ml
PK-CT-2000-OLI-500	PromoFectin-Oligo	5 x 1 ml

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