

METAFECTENE®

The efficient transfection reagent for mammalian cells



Highlights

- ▶ Suitable for DNA transfection
- ▶ High transfection efficiency
- ▶ Free from serum inhibition
- ▶ Low cytotoxicity, transfection solution can generally remain on the cells
- ▶ Comprehensive library of user reports
- ▶ Wild range of references in specialist literature

Technology

METAFECTENE® is a liposome-based transfection reagent composed of a mixture of polycationic and neutral lipids, which enables efficient levels to be reached in DNA transfection of most mammalian cells.

In addition, low cytotoxicity and freedom from serum inhibition make it suitable for a wide range of uses. In comparison with first-generation transfection reagents reagents of the second generation provide for structure elements owing so called "proton-sponge" activity. As a consequence of "endosome buffering" nucleic acids are released more easily to the cytosol, thus increasing the efficiency of transfection.

Product Specifications

Application	Transfection of mammalian cells with nucleic acids
Formulation	Cationic lipids with colipids in water
Assays	Up to 1500 (24-well) or up to 400 (6-well) per 1 ml reagent

Order Numbers

Product	Order-No.	Size	CHF
Test sample			
METAFECTENE®	T020-0.2	200µl	0.-
METAFECTENE®	T020-1.0	1.0ml	240.-
METAFECTENE®	T020-2.0	2x1.0ml	899.-
METAFECTENE®	T020-5.0	5x1.0ml	1611.-

Metafectene® Technical Note for Lentiviral Production

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Virus production: Transfection of 293T/17 cells for production of lentiviruses

The here described procedure for production of lentiviruses extends over four days plus three more days for determining the viral titer as described in [1].

Material

Plasmid pHR'SINcPPT-SEW carrying EGFP as transgene controlled by the SFFV promoter

Plasmid pCMVΔR8.91 packaging plasmid containing the HIV genes gag and pol

Plasmid pMD.G encoding the envelope protein VSV-G

Metafectene Biontexas T020-5.0

Na-butyrate Sigma Aldrich B-5887
1 M stock solution in PBS

Poly-D-Lysine Sigma P-7280
1 mg/ml stock solution in PBS

DMEM with FCS and P/S

DMEM without additives

Millex-Ha Filter 0.45 μm
Millipore SLHA 033SS

Vivaspin 100 000 MW cut-off
Sartorius VS2041

The lentiviral vector system

The lentiviral plasmid pHR'SINcPPT-SEW [2] contains the information of the recombinant lentiviral genome which is devoid of any information for the original HIV proteins. Instead, flanked by the 5'LTR (long terminal repeat domain) and the Δ3'LTR (with deleted promoter sequences in order to reduce the risk of activation of downstream genes after integration into the human genome) the transgene EGFP (enhanced green fluorescent protein) is placed under the control of the U3 region of the LTR of SFFV (spleen focus-forming virus) and upstream of the WPRE (woodchuck hepatitis virus posttranscriptional regulatory element). An additional element is the packaging signal Ψ which is required that the viral RNA will be used as viral genome and packed into the viral capsid. The capsid protein and the reverse transcriptase are encoded by the genes gag and pol on the packaging plasmid pCMVΔR8.91. The third plasmid delivers the information for the envelope protein VSV-G (vesicular stomatitis virus glycoprotein) for pseudotyping (enlargement of the spectrum of transducible cell types even of different species) of the viral capsid. This is a packaging system of the second generation (for further information about lentiviral vector systems see [3]).

Protocol

All information is for a culture dish of a diameter of 10 cm, and all solutions should be warmed up to room temperature.

Day 1:

The culture dish is coated with 3 ml Poly-D-Lysine (0.1 mg/ml in PBS). After 5 min, Poly-D-Lysine is removed and stored at 4 °C since it can be used several times. The dish is washed with 5 ml sterile water and thus ready for use.

5×10^6 293T/17 cells are seeded in 14 ml DMEM with additives and cultured overnight.

Day 2:

On the second day transfection is performed with three plasmids.

Overall 10 µg of DNA is used for one culture dish: 4.4 µg lentiviral plasmid, 3.4 µg pCMVΔR8.91, and 2.2 µg pMD.G. The plasmids are given into a well of a 24-well plate and mixed thoroughly with 700 µl DMEM without additives → solution A.

46 µl Metafectene is mixed in another well of a 24-well plate with 700 µl of DMEM without additives → solution B.

Then solution A and B are mixed in one well and incubated for 20 min at room temperature. The solution of nearly 1.5 ml is added drop by drop to the cells with concomitant gentle orbital shaking of the dish. This is followed by an overnight incubation at 37 °C.

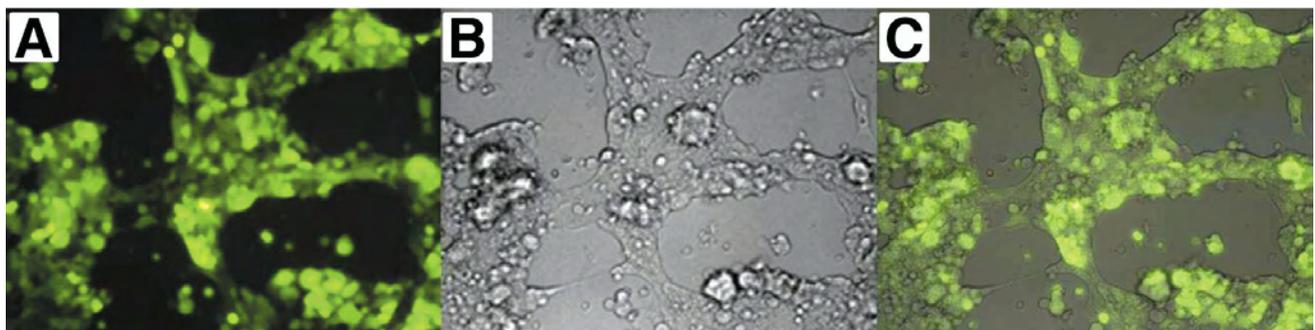
Day 3:

In the morning, the medium is replaced by 14 ml DMEM with additives and 140 µl Na-butyrate (10 mM).

In the evening the medium is replaced by 7 ml DMEM without additives.

Day 4:

The medium is collected (it can be stored at 4 °C for several hours) and filtered with a 0.45 µm filter. The cleared virus-containing solution is put on a Vivaspin-tube and concentrated up to 0.5 ml or 1 ml final volume (depending on the number of 10 cm dishes used for virus production) with 3000 rpm (\approx ca. 1800g) at 4 °C in an Eppendorf tabletop centrifuge. Aliquots of 50 µl or 100 µl are stored at -70 °C.

**Figure 1.**

EGFP-expressing cells two days after transfection. Overlay (C) of the fluorescent channel (A) with the bright field picture (B) illustrates that a nearly 100 % transfection efficiency was achieved with the transfection conditions as described in our protocol.

Virus titration: Transduction of HT1080 cells

Material

24 well plate

Polybrene hexadimethrine bromide
Sigma H-9268

Polybrene working solution
800 µg/ml PBS sterile filtrated,
storage at 4°C for months

PBS without Ca/Mg
Gibco 14190-094

DMEM Gibco 41965-039

Protocol

All work must be done under S2 conditions.

Day 1:

8×10^4 HT 1080 cells are seeded per well of a 24-well plate. Titration of each dilution should be done in duplicates or triplicates!

Day 2:

Four dilutions are analysed: 1:100, 1:1000, 1:10000, 1:100000.

1. dilution (1:100):

10 µl viral supernatant + 980 µl medium +
10 µl Polybrene working solution

Further dilutions:

100 µl of the previous dilution + 890 µl Me-
dium + 10 µl Polybrene working solution

The medium on cells is replaced by 300 µl of
each dilution.

Day 3:

The medium is exchanged with 0.5 ml DMEM
without Polybrene per well.

Day 4 or 5:

The cells are harvested, washed once with
1 ml PBS (centrifuge 3 min with 3000 rpm \pm
ca. 1800g in an Eppendorf tabletop centri-
fuge) and $0.3-1 \times 10^6$ cells are resuspended
in 300 µl staining medium for FACS.

Analysis

Example: 80000 cells \pm 100 %

FACS result:

22.5 % GFP-positive cells

22.5 % transduction efficiency

supernatant diluted 1:1000 in 300 µl

=> amount of viral particles in 1 ml concen-
trated supernatant = (transduction efficiency
x total cell number)/100 % x (1 ml/volume
of medium containing diluted viral particles)
x dilution factor

= (22.5 % x 80000) / 100 % x (1000 µl/300
µl) x 1000

= 22.5 x 8000/3 x 1000

= 6×10^7 infectious particles (titer) per 1 ml

References

1. Maier, P.; Herskind, C.; Fleckenstein, K.; Spier, I.; Laufs, S.; Zeller, W. J.; Fruehauf, S.; Wenz, F., MDR1 gene transfer using a lentiviral SIN vector confers radioprotection to human CD34+ hematopoietic progenitor cells. *Radiat Res* 2008, 169, (3), 301-10.
2. Demaison, C.; Parsley, K.; Brouns, G.; Scherr, M.; Battmer, K.; Kinnon, C.; Grez, M.; Thrasher, A. J., High-level transduction and gene expression in hematopoietic repopulating cells using a human immunodeficiency [correction of imunodeficiency] virus type 1-based lentiviral vector containing an internal spleen focus forming virus promoter. *Hum Gene Ther* 2002, 13, (7), 803-13.
3. Maier, P.; von Kalle, C.; Laufs, S., Retroviral vectors for gene therapy. *Future Microbiol* 2010, 5, 1507-23.