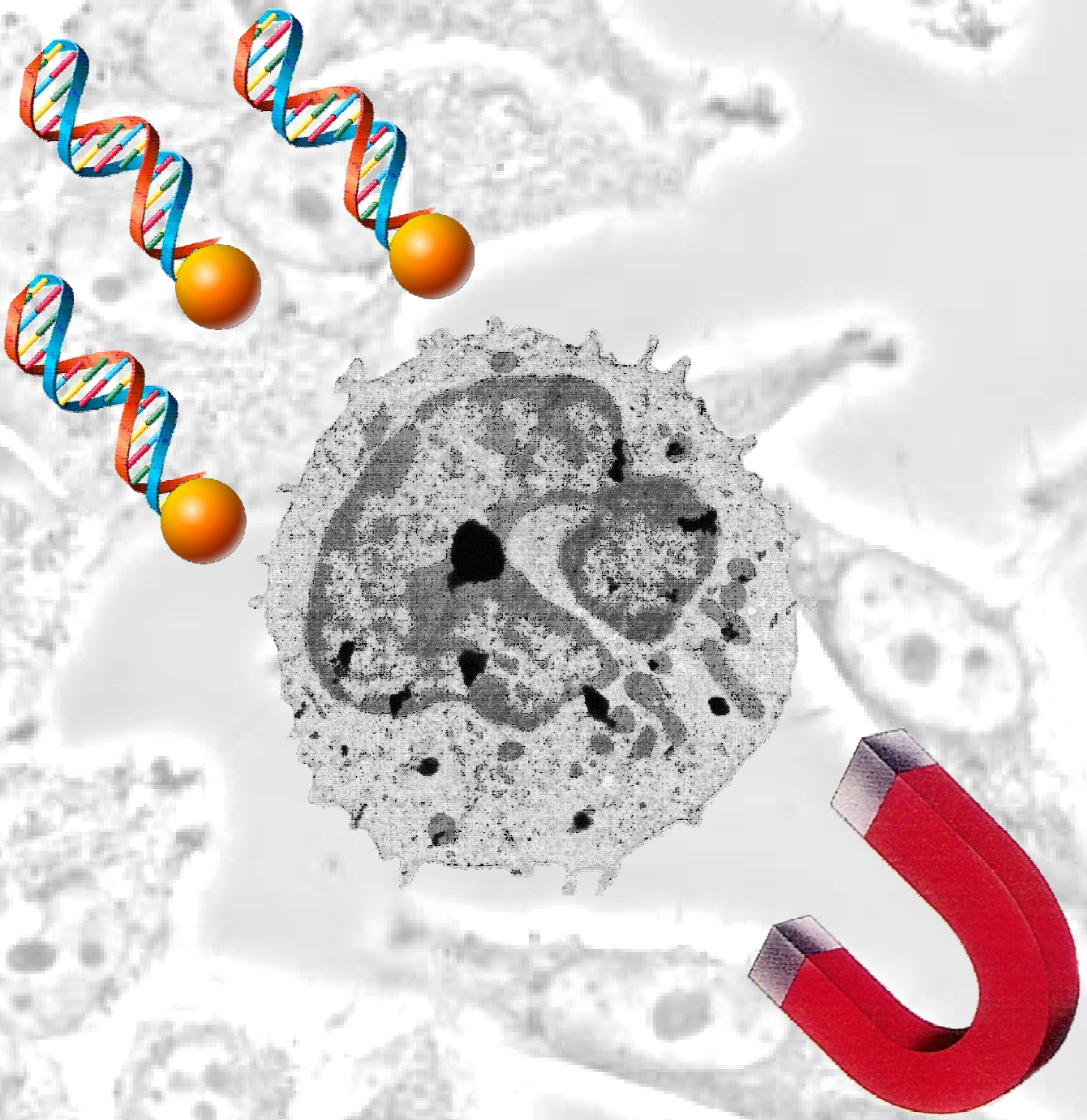
Magnetofection™

the new gene transfection technology

Magnetofection™ is a novel, simple and highly efficient method to transfect cells in culture.



chemicell

NEW TOOLS IN BIOSCIENCES

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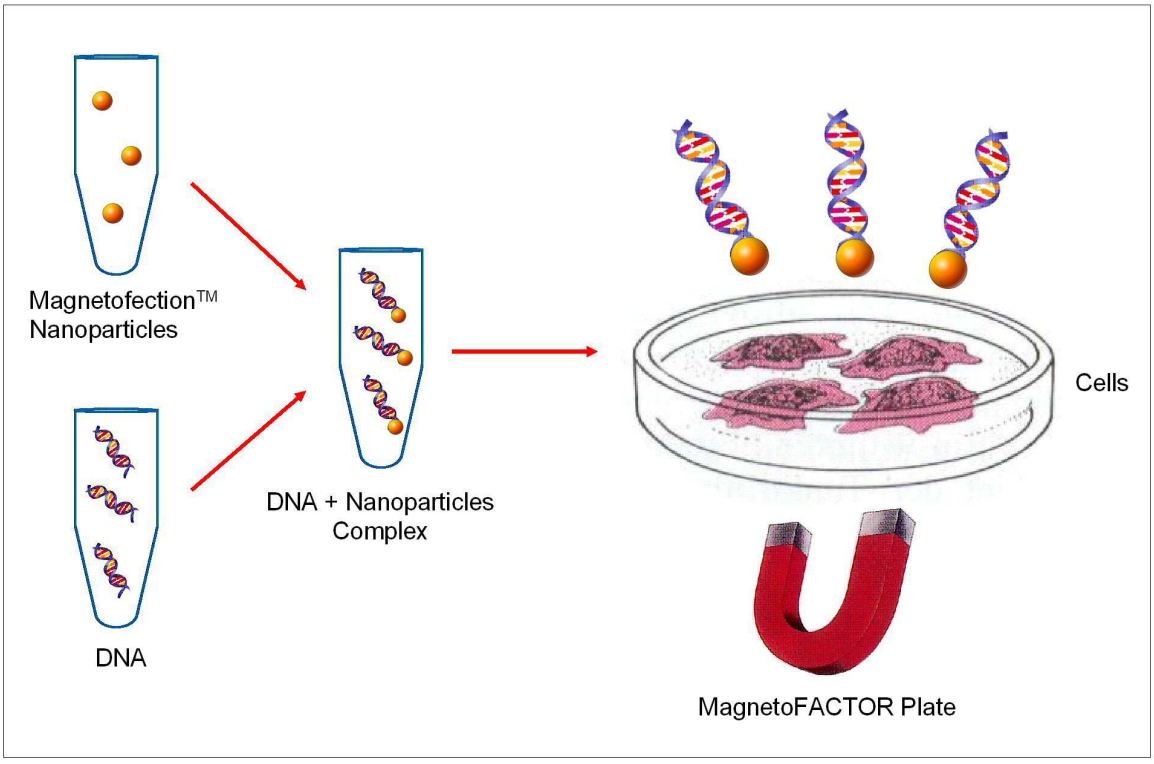
1.1. Description

Magnetofection™ is a novel, simple and highly efficient method to transfect cells in culture. It exploits magnetic force exerted upon gene vectors associated with magnetic particles to draw the vectors towards, possibly even into, the target cells. In this manner, the full vector dose applied gets concentrated on the cells within a few minutes so that 100% of the cells get in contact with a significant vector dose. This has several important consequences:

- *Greatly improved transfection rates in terms of percentage of cells transfected compared to standard transfection.*
- *Up to several thousand fold increased levels of transgene expression compared to standard transfections upon short-term incubation.*
- *High transfection rates and transgene expression levels are achievable with extremely low vector doses, which allows to save expensive transfection reagents.*
- *Extremely short process time. A few minutes of incubation of cells with gene vectors are sufficient to generate high transfection efficiency, compared to several hours with standard procedures.*



1.2. Magnetofection™ Reagents

As the manufacturer of the Magnetofection™ technology, **chemicell** offers two types of ready-to-use Magnetofection™ reagents.

PolyMAG is a universally applicable magnetic particle preparation for high efficiency nucleic acid delivery. It is mixed in a one-step procedure with the nucleic acid to be transfected and has been used successfully with plasmid DNA, antisense oligonucleotides and siRNAs.

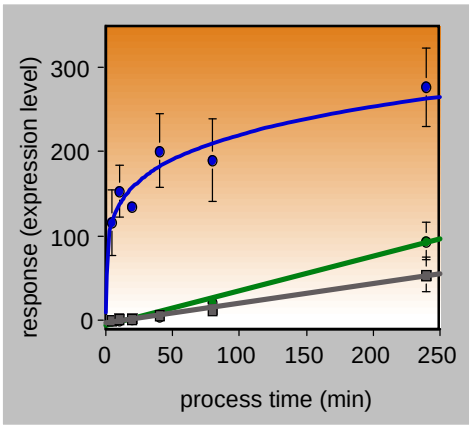
CombiMAG is a magnetic particle preparation designed to be combined with any commercially available transfection reagent such as polycations and lipids and can be associated with plasmid DNA, antisense oligonucleotides, siRNAs or viruses.
It allows you to create your own magnetic gene vector based on your favourite transfection reagent.

Purchaser Notification

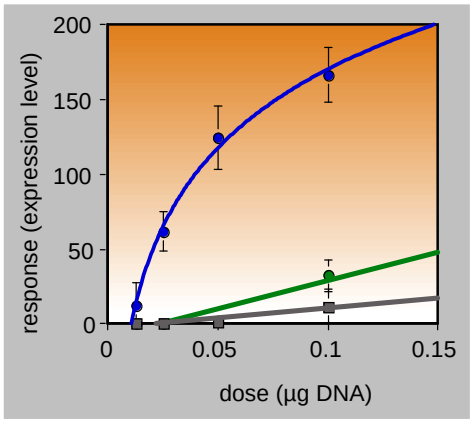
The Magnetofection™ reagents and all of its components are developed, designed, intended and sold for research use only. They are not to be used for human diagnostic or any drug intended.

Magnetofection™ are registered trademark

1.3. Nuclec Acids Dose Response and Transfection Kinetics



Transfection kinetics: NIH 3T3 cells were incubated with GenePorter™ (Gene Therapy Systems) ± CombiMAG with and without positioning on the Magneto-FACTOR plate for the indicated time spans. Luciferase expression was assayed after 24 hours.



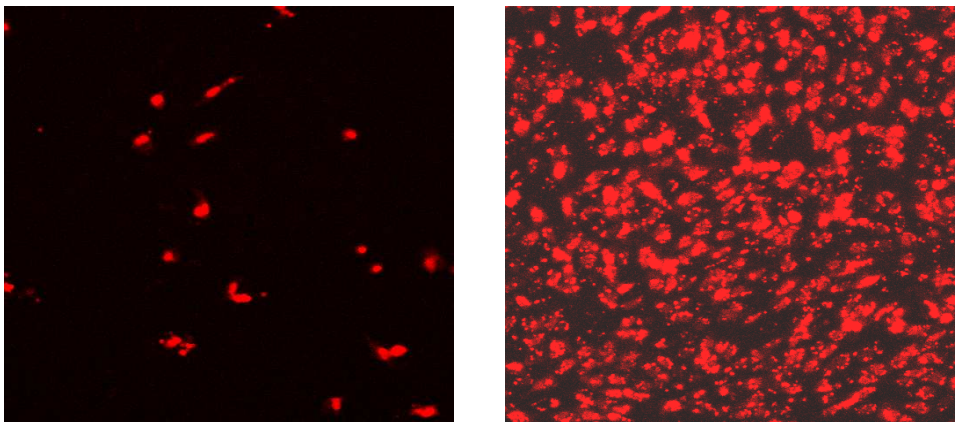
Dose response profile in NIH 3T3 cells using Lipofectamine™ (Invitrogen) ± CombiMAG with and without positioning on the MagnetoFACTOR plate for 15 min. Luciferase expression was assayed after 24 hours.

1.4. Application

Magnetofection™ is generally applicable for adherent cells and has been tested with a variety of immortalized cell lines and primary cells listed below. If a particular cell type or cell line is not listed this does not mean that Magnetofection™ would not work. Also for the cells listed, some reagents have not been tested so far, as indicated by “n.d.” (not determined).

The **CombiMAG** reagent can be combined with any polycationic and lipidic transfection reagent, and also with adenoviral and retroviral vectors. In some cases, references are made in the footnotes to very successful combinations with commercially available reagents that have been tested so far.

Type of nucleic acid / virus	PolyMAG	CombiMAG
Plasmid DNA	√	√
Antisense Oligonucleotides	√	√
siRNA	√	√
Adenovirus	n.d.	√
Retrovirus	n.d.	√



Primary human umbilical vein endothelial cells positioned on the MagnetoFACTOR plate were incubated for 15 min with a Cy3 fluorescence-labeled antisense-oligonucleotide complexed with Effectene™ (Qiagen; left) or Effectene™ + CombiMAG (right).

(Data kindly provided by F. Kroetz. Ludwig-Maximilians University Munich)

Adherent Cells					
Cell line	**Cell line**	**Source**	**PolyMAG**	**CombiMAG***	**Ref. No.**
HeLa	cervix carcinoma	human	+	+	9.030, 9.024, 9.072, 9.012, 9.111, 9.003, 9.005, 9.016,
HEK 293	kidney	human	+	+	9.013, 9.064, 9.054,
BHK	kidney	hamster	n.d.	+	-
CHO	ovarian	hamster	+	+	9.036, 9.002, 9.005, 9.006,
NIH 3T3	fibroblasts	mouse	+	+	9.003, 9.072, 9.091, 9.002, 9.005, 9.004,
16HB140	airway epithelium	human	+	n.d.	9.010,
EJ28	bladder cancer	human	+	n.d.	-
HBL-100	breast	human	n.d.	+	-
MCF7	breast adenocarcinoma	human	+	+	9.014, 9.040, 9.041, 9.115,
MCS7	breast adenocarcinoma	human	+	n.d.	-
HCT 116	colon adenocarcinoma	human	n.d.	+	-
U373	astrocytoma	human	n.d.	+	9.056,
U87	glioblastoma	human	n.d.	+	9.056,
U251	glioblastoma	human	n.d.	+	9.056,
T98G	glioblastoma	human	n.d.	+	9.056,
YK6-1	glioblastoma	human	n.d.	+	9.056,
YH-13	glioblastoma	human	n.d.	+	9.056,
A549	alveolar basal epithelial	human	+	+	9.075, 9.101,
A172	glioblastoma	human	n.d.	+	9.056, 9.074,
LN229	glioblastoma	human	+	+	9.074,
LN18	glioblastoma	human	+	+	9.074,
SW480	colon adenocarcinoma	human	n.d.	+	-
LoVo	colon adenocarcinoma	human	n.d.	+	-
HCT15	colon adenocarcinoma	human	n.d.	+	-
CEMx174	hybrid T/B-lymphocytes	human	n.d.	+	9.060,
CHO	ovarian	human	+	+	-

A431	epidermoid carcinoma	human	n.d.	+	-
HT-1080	fibrosarcoma	human	+	n.d.	-
HFF	foreskin fibroblast	human	+	+	-
Hep G2	hepatocellular carcinoma	human	+	+	9.012, 9.048,
HaCat	keratinocytes	human	n.d.	+	-
293 T	kidney	human	+	+	9.030,
293 T-17	kidney	human	n.d.	+	9.030,
SK-MES-1	lung carcinoma	human	+	n.d.	-
MRC 5	lung, embryonic	human	+	+	-
MeWo	melanoma	human	n.d.	+	-
NYGM	cerebral glioblastoma	human	n.d.	+	9.056,
SK-MEL-28	melanoma	human	n.d.	+	-
MEP	mammalian epithelial	human	n.d.	+	9.047,
MOLT-4	acute lymphoblastic leukemia	human	n.d.	+	9.044,
SHSY-5Y	neuroblastoma	human	n.d.	+	9.111, 9.050,
SV40	transformed fibroblasts	human	+	n.d.	9.027,
A549	Non-small cell lung carcinoma	human	n.d.	+	9.057, 9.101
SaOS-2	osteo sarcoma	human	n.d.	+	-
BTK-143	osteo sarcoma	human	n.d.	+	-
SaOS-2	osteo sarcoma	human	n.d.	+	-
BEAS-2B	lung epithelial cells	human	+	n.d.	9.012
181RDB	pancreatic	human	n.d.	+	-
HN12	Head, neck carcinoma	human	n.d.	+	9.028
PC-3	prostate carcinoma	human	n.d.	+	-
H9	lymphoblastoid	human	n.d.	+	9.077
H295R	adrenocortical carcinoma	human	n.d.	+	9.058, 9.059, 9.037
HOS	osteosarcoma	human	n.d.	+	9.077
HSG	salivary gland, submandibular.	human	n.d.	+	-
He99	lung cancer	human	+	+	9.100
HMEC-1	microvascular endothelial	human	+	n.d.	9.038, 9.092

NCI-H441	lung adenocarcinoma epithelial	human	+	+	9.073
NCI-H292	mucoepidermoid carcinoma	human	+	n.d.	9.065, 9.095, 9.112
NCI-H82	small cell lung cancer	human	+	n.d.	-
SK-N-BE2	neuroblastoma	human	+	n.d.	9.031
MDAMB231	breast adenocarcinoma	human	+	n.d.	9.035
ECV-304	urinary bladder carcinoma	human	n.d.	+	-
11.5dpcXY	gonad explants	mouse	n.d.	+	9.109
Cal27	oral adenosquamous carcinoma	mouse	n.d.	+	9.028
CT-26	colon carcinoma	mouse	+	n.d.	9.003, 9.005
C127	murine mammary tumor	mouse	n.d.	+	9.042
F9	embryonal carcinoma	mouse	+	n.d.	-
L929	fibroblast, connective tissue	mouse	+	n.d.	9.016
MEF	fibroblast	mouse	+	n.d.	9.039, 9.098
HT-22	hippocampal neuroblast	mouse	n.d.	+	-
mIEnd	Mesenteric lymph node	mouse	n.d.	+	9.085
mIC-(cl2)	intestine	mouse	n.d.	+	-
B16F10	melanoma	mouse	+	n.d.	9.076, 9.005
N2a	neuroblastoma	mouse	+	n.d.	9.063, 9.086
NS20Y	neuroblastoma	mouse	n.d.	+	-
bTC-tet	B-cell line	mouse	+	+	9.020
Hepa1c1c7	murine hepatoma	mouse	+	n.d.	9.084
SVEC	murine cell line	mouse	n.d.	+	9.028
OP9	bone marrow-derived stromal	mouse	n.d.	+	9.047
SM 10	trophoblast	mouse	n.d.	+	-
STC-1	enteroendocrine I cells	mouse	+	+	9.033
A7r5	aortic smooth muscle	rat	n.d.	+	9.094
PC12	adrenal pheochromocytoma	rat	+	n.d.	-
PC6-3	daughter cell line of PC12	rat	+	n.d.	-
C6	glioma	rat	n.d.	+	-

H4IIE	hepatoma	rat	n.d.	+	9.062
L6	skeletal muscle cell	rat	+	n.d.	-
RIE1-α5	intestinal	rat	+	n.d.	9.088
HNSCC	Head, neck carcinoma	rat	n.d.	+	9.015, 9.028, 9.051,
RV	vein	rat	+	n.d.	-
COS-7	kidney	monkey	+	+	9.030
CV-1	kidney	monkey	+	n.d.	-
COS-1	kidney	monkey	n.d.	+	-
Vero	kidney	monkey	n.d.	+	9.045, 9.046, 9.016
B11	(UV) resistant ovary	hamster	n.d.	+	9.028
V79	lung fibroblasts	hamster	+	n.d.	-
PT-11	kidney	bovine	n.d.	+	-
MDCK	kidney	canine	+	n.d.	9.067
CRFK	kidney	cat	n.d.	+	-
VSa13	bone (chondrocyte-like)	fish	+	n.d.	-
AM-C6SC8	kidney	pig	n.d.	+	-
-	fibroblasts	xenopus	+	n.d.	-
EMC	mesenthelial. Cells (epicard)		n.d.	+	-

Suspension Cells					
Cell line	**Cell type**	**Source**	**PolyMAG**	**CombiMAG***	**Ref. No.**
THP-1	acute myeloid leukemia	human	n.d.	+	-
Jurkat	acute T-cell lymphoma	human	n.d.	+	9.044, 9.073
BL-41	B-cell lymphoma	human	+	n.d.	-
K562	chronic myeloid lymphoma	human	n.d.	+	9.002
P815	mastocytoma	mouse	n.d.	+	-
U937	histiocytic lymphoma	human	+	+	-

Primary Cells				
Cell type	**Source**	**PolyMAG**	**CombiMAG***	**Ref. No.**
endothelial cells (cord blood)	human	+	n.d.	9.054
aortic endothelial cells	human	+	n.d.	9.028, 9.032
epithelial (HUVEC)	human	+	+	9.029, 9.034, 9.092, 9.113, 9.114, 9.003, 9.007, 9.008, 9.021, 9.029, 9.034, 9.005, 9.004
fibroblasts	human	+	+	-
fibroblasts, diploid	human	+	+	-
gastric gland	human	+	+	-
adherent gastric cells	human	n.d.	+	9.069
gastric myofibroblasts	human	+	n.d.	9.043
glioma	human	+	n.d.	-
keratinocytes	human	n.d.	+	9.005
LNCaP	human	n.d.	+	-
lymphocytes	human	+	+	9.005, 9.102
mammal epithelium	human	n.d.	+	-
nasal airway epithelium	human	+	+	9.005
pancreatic tumor	human	+	n.d.	-
peripheral blood lymphocytes	human	+	+	9.005, 9.102
stroma cells (endometrium)	human	n.d.	+	-
T-cells	human	+	+	9.102, 9.089
chondrocytes	human	+	+	9.107, 9.022, 9.024
trophoblastic cells	human	n.d.	+	-
fibroblasts (MEF)	mouse	n.d.	+	9.018, 9.053
neurons	mouse	+	+	9.071
vagal afferent neurons	mouse	n.d.	+	9.071
adherent gastric cells	mouse	n.d.	+	9.061
peripheral blood lymphocytes	mouse	n.d.	+	-
myoblasts	mouse	n.d.	+	9.053
T-cells	mouse	+	n.d.	-

hippocampal neurons	rat	+	+	9.017, 9.070, 9.050, 9.052, 9.093, 9.090, 9.105, 9.104
aortic smooth muscle cells	rat	n.d.	+	9.094
cardiomyocyte	rat	n.d.	+	9.055
hepatocytes	rat	n.d.	+	-
epithelial (HUVEC)	rat	+	+	9.028, 9.054
cortical neurons	rat	n.d.	+	9.052, 9.063, 9.097
aortic endothelial cells	bovine	+	n.d.	-
carotid artery smooth muscle	bovine	+	n.d.	-
chromaffine cells	bovine	n.d.	+	-
lens	bovine	+	n.d.	-
airway epithelium	pig	n.d.	+	-
chondrocytes	pig	+	+	-
fibrochondrocytes	pig	+	+	-
pSM, smooth muscle	pig	n.d.	+	-

* Corresponds to successfully transfected by CombiMAG in association with different commercial transfection reagents such as FuGENE™ (Roche), Lipofectamine™, METAFECTENE™ (Biontex GmbH) and DMRIE-C™ (Invitrogen); adenovirus; Effectene™ (Qiagen)

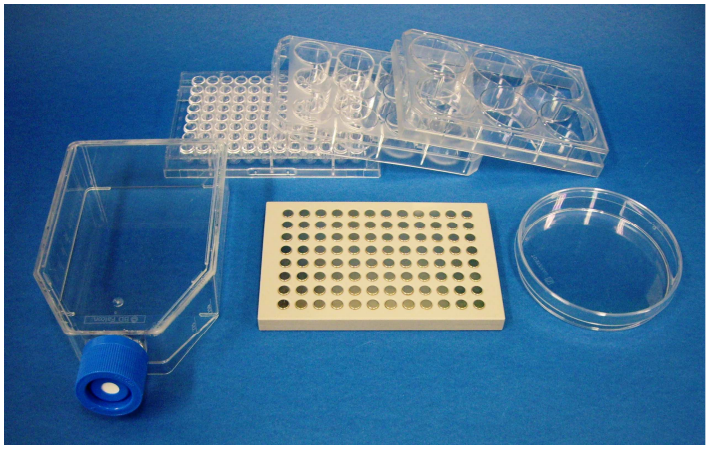
n.d. = not determined

2.1. MagnetoFACTOR plates

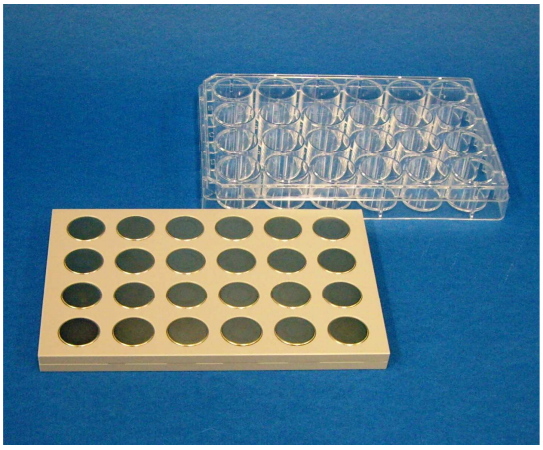
Apart from suitable magnetic nanoparticles, Magnetofection™ requires appropriate magnetic fields. These are provided by the MagnetoFACTOR plate, especially designed for Magnetofection™.

The MagnetoFACTOR-96 plate its special geometry not only produces strong magnetic fields under each well of 96-well plates but is also applicable for other plate formats T-75 culture flasks, 6- and 12-well plates. In the larger plate formats, the MagnetoFACTOR plate will produce a pattern of higher and lower densities of transfected cells according to the geometry of the magnetic field lines. The MagnetoFACTOR-24 plate is special designed for the 24-well format.

The MagnetoFACTOR plates are compatible for well plates of the most manufacturer.



MagnetoFACTOR-96 plate



MagnetoFACTOR-24 plate

SPECIAL OFFER

As an introductory offer you will receive one vial each of PolyMAG and CombiMAG sufficient for 200 µg of DNA each for free in combination with the purchase of the MagnetoFACTOR plate. This will enable you to optimize your Magnetofection™ in up to 3000 transfection experiments (96-well format).

@ info@chemicell.com

Avalaible Kits

3.1. Kits Contents

Product Number	Description	Size (sufficient for n µg DNA)	Number of Transfection (96-well format)	Price EUR / USD
9001	PolyMAG-100	100	1000	95 / 125
9002	PolyMAG-500	500	5000	365 / 480
9003	PolyMAG-1000	1000	10000	665 / 875
9004	CombiMAG-100	100	500	50 / 65
9005	CombiMAG-500	500	2500	180 / 235
9006	CombiMAG-1000	1000	5000	350 / 460
9007	MagnetoFACTOR-96 plate	-	-	350 / 460
9009	MagnetoFACTOR-24 plate	-	-	350 / 460
9008-96	**SPECIAL OFFER:**			**350 / 460**
	MagnetoFACTOR-96 plate	-	-	
	PolyMAG-200	200	2000	
	CombiMAG-200	200	1000	
9008-24	**SPECIAL OFFER:**			**350 / 460**
	MagnetoFACTOR-24 plate	-	-	
	PolyMAG-200	200	2000	
	CombiMAG-200	200	1000	

Storage:
All components of the Magnetofection™ kit should be stored at room temperatur (20-25°C). After first use store the kit at +4°C.

- Do not freeze the magnetic nanoparticles
- Do not add anything to the stock solution of magnetic nanoparticles
- Shipping Conditions: Room Temperatur

3.2. Contact

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4.1. General Considerations

The instructions given below represent sample protocols that were applied successfully with a variety of cell lines. **Please note** that optimal conditions do vary from cell line to cell line. Therefore, the amount of DNA or RNA used and the ratios of the individual components may have to be adjusted to achieve best results. The following recommendations can be used as guidelines to achieve good transfection with minimal incubation times.

4.2. General Protocol

Adherent cells are seeded such that they reach 60-80% confluency at the time of Magnetofection™.

For **suspension cells**, use the specific protocol given in section 4.6. Immediately preceding transfection, the medium can be replaced with fresh medium (optionally without serum) if necessary.

The suggested cell number for adherent and suspension cells is given below.

Recommended DNA amount, PolyMAG volume and transfection volume.

Tissue Culture Dishes	Cell Number per well	DNA Quantity (µg)	PolyMAG or CombiMAG (µL)	Transfection Volume*
96-well	0.5 - 2 x 10^4	0.1 - 0.5	0.1 - 0.5	200 µL
24-well	0.5 - 1 x 10^5	0.5 - 2	0.5 - 2	500 µL
12-well	1 - 2 x 10^5	2 - 4	2 - 4	1 mL
6-well	1 - 4 x 10^5	2 - 6	2 - 6	2 mL
60 mm dish	5 - 10 x 10^5	6 - 8	6 - 8	5 mL
90-100 mm dish	1 - 2 x 10^6	8 - 12	8 - 12	10 mL
T-75 flask	2 - 5 x 10^6	15 - 25	15 - 25	15 - 20 mL

*Total Transfection volume = culture medium + PolyMAG or CombiMAG complex

The following protocols (section 4.4, 4.5, 4.5.1, 4.5.2 and 4.6) can be used to produce stably transduced cells except that 48 hours post transfection. Cells are transferred to fresh medium containing the appropriate antibiotics for selection. It is important to wait at least 48 hours before exposing the transduced cells to selection media.

Vectors are prepared in medium without serum and supplement or in physiological saline, because serum may interfere with transfection complex assembly. The serum and supplement-free transfection complex is added to the cells that are covered with complete medium. Therefore, the addition of the transfection complex will result in the dilution of supplements such as serum, antibiotics or other additives of your standard culture medium. Although a medium changes after Magnetofection™ is not required for most cell types, it may be necessary for cells that are sensitive to serum/supplement concentration. Alternatively, the cells may be kept in serum-free medium during Magnetofection™. In this case, a medium change will be required after Magnetofection™.

4.3. Magnetofection™ in 6-, 12- , 24- or 96-well Plate Formats, T-75 Culture Flasks

As a rule of thumb, a DNA amount of 50 ng to 300 ng per square centimeter culture dish will yield good results. However, it is emphasized that every cell line requires optimization with respect to DNA amount and vector formulation.

The easiest way to generate the complexes is to provide the required amount of magnetic particles in a microcentrifuge tube, add the required amount of DNA which has been diluted with **serum-free medium** (e.g. DMEM). After 15 min incubation, add the magnetic particles / DNA complex to the cells. Position the 6-well culture plate on the **MagnetoFACTOR** plate for up to 10-20 min, subsequent perform a medium change (optional).

6-well culture dish:

A useful DNA amount for the 6-well format is 2 µg up to 6 µg, whereas the ratio of magnetic particles / DNA is 1:1. To 1.8 mL cells per well add **200 µL** magnetic particles / DNA complexes.

12-well culture dish:

A useful DNA amount for the 12-well format is 2 µg up to 4 µg, whereas the ratio of magnetic particles / DNA is 1:1. To 0.8 mL cells per well add **200 µL** magnetic particles / DNA complexes.

24-well culture dish:

A useful DNA amount for the 24-well format is 0.5 µg up to 2 µg, whereas the ratio of magnetic particles / DNA is 1:1. To 0.3 mL cells per well add **200 µL** magnetic particles / DNA complexes.

96-well culture dish:

A useful DNA amount for the 96-well format is 0.1 µg up to 0.5 µg, whereas the ratio of magnetic particles / DNA is 1:1. To 0.15 mL cells per well add **50 µL** magnetic particles / DNA complexes.

T-75 culture flask:

A useful DNA amount for the T-75 culture flask format is 15 µg up to 25 µg, whereas the ratio of magnetic particles / DNA is 1:1. To 14 mL (19 mL) cells add **1 mL** magnetic particles / DNA complexes.

4.4. PolyMAG

Plate the adherent cells the day before transfection or suspension cells just before transfection in the appropriate tissue culture dish and volume of culture medium as recommended in table section 4.2.

The protocol is as simple as follows: Use 1 µL of **PolyMAG** per µg of DNA.

1. Add the required amount of **PolyMAG** (according to the DNA amount) to a microcentrifuge tube or to a U-bottom well of 96-well plate. If required and for amount less than 1 µL **PolyMAG** in your protocol, predilute **PolyMAG** with deionized water.

Note: *Vortex the* ***PolyMAG*** *before used.*

2. Dilute required amount of DNA to 200 µL with **serum- and supplement-free medium** (such as DMEM).

3. Add the 200 µL DNA solution to **PolyMAG** and mix immediately by vigorous pipetting.

4. After 15 minutes of incubation, add the 200 µL of complexes to the cells.

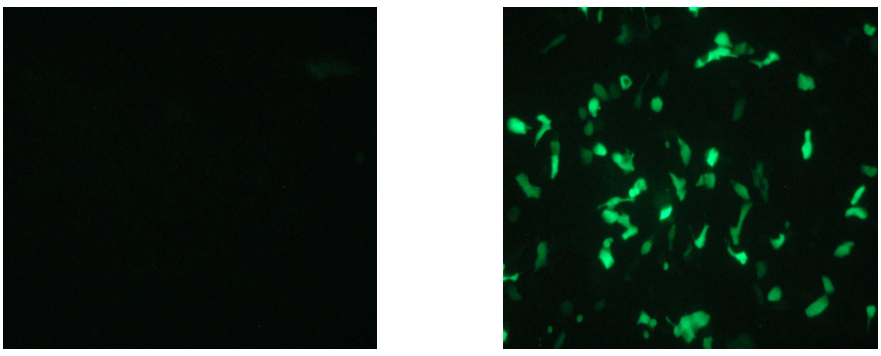
Note: *The total transfection volumes per well (culture medium +* ***PolyMAG*** *complex) are suggested in the table above.*

5. Place the cell culture plate upon the **MagnetoFACTOR** plate and incubate under standard cell conditions for 10 to 20 minutes.

6. Remove the **MagnetoFACTOR** plate.

6a. Optionally perform a medium change.

7. Cultivate cells under standard conditions until evaluation of transgene expression.



STANDARD TRANSFECTION **MAGNETOFECTION™**

Confluent primary human Keratinocytes transfected with PolyMAG

4.5. CombiMAG

A number of suppliers sell efficient transfection reagents. All of these can be made a magnetofectin by simple mixing with **CombiMAG**, usually resulting in strong improvements of these reagents efficiencies.

There are two strategies of using **CombiMAG**:

- One is to prepare a standard DNA complex with a commercial transfection reagent according to the instructions of the manufacturer, followed by mixing with **CombiMAG**.

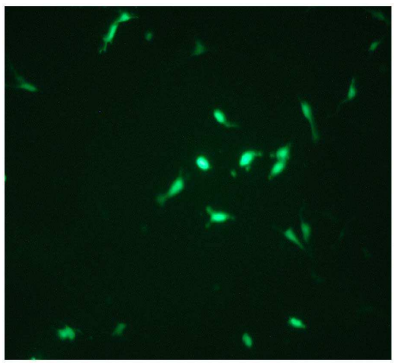
- The second strategy is to first mix DNA and **CombiMAG** followed by mixing with the transfection reagent.

Also in this case, the instructions of the manufacturer are used with the only exception that instead of DNA alone, a mixture of DNA and **CombiMAG** is added to the transfection reagent.

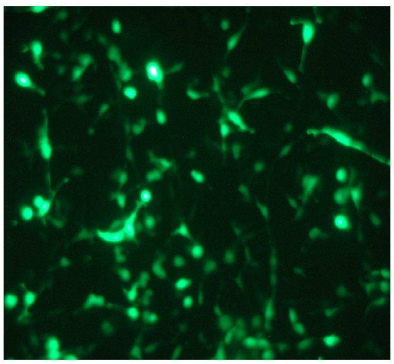
Depending on the transfection reagent, the mixing order of components may influence the final transfection efficiency. It is recommended to use 1 - 2 µL of **CombiMAG** per µg of DNA in initial experiments.

However, depending on the cell line to be transfected and the commercial transfection reagent used, the optimal composition may be found above or below this ratio.

Note: *If required in your setup, predilute* ***CombiMAG*** *with ddwater.*



CT-26 colon carcinoma cells were transfected for 15 min with a GFP reporter plasmid complexed with DMRIE-C (Invitrogen)



CT-26 colon carcinoma cells were transfected for 15 min with a GFP reporter plasmid complexed with DMRIE-C (Invitrogen) + CombiMAG on a magnetic plate

(Data kindly provided by Ch. Plank. Technical University Munich)

4.5.1 CombiMAG - Example Protocol for Fugene (Roche)

Plate 0.5 x 10^5 adherent cells per well the day before transfection or suspension cells just before transfection in a 24-well culture plate.

1. Add 2.4 µL of Fugene to 97.6 µL **serum- and supplement-free medium** (such as DMEM) and vortex vigorously for 2 seconds.

2. Dilute 0.8 µg of DNA to 100 µL with **serum- and supplement-free medium** (such as DMEM).

3. Mix the DNA solution with the Fugene dilution by pipetting and incubate for 15 minutes at room temperature.

4. Add the resulting 200 µL of DNA complex to 1.6 µL of **CombiMAG** and mix immediately by vigorous pipetting. Vortex the **CombiMAG** before used.

5. After further 15 minutes of incubation add the DNA / Fugene / **CombiMAG** complex to the cells.

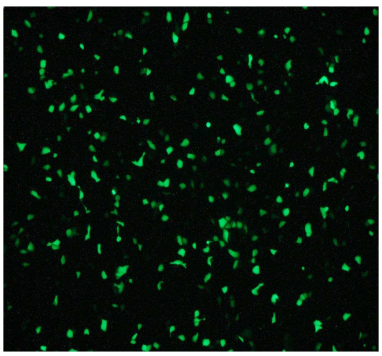
6. Place the cell culture plate upon the **MagnetoFACTOR** plate and incubate under standard cell culture conditions for 10 to 20 minutes.

7. Remove the magnetic plate and cultivate cells under standard conditions until evaluation of transgene expression.

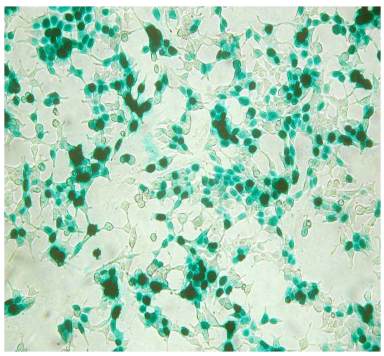
8. Optionally perform a medium change.

9. Depending on the commercial transfection reagent used, this protocol may have to be adapted.

Note: *If required in your setup, predilute* ***CombiMAG*** *with ddwater.*



Human Keratinocytes



Porcine Chondrocytes

Transfection of primary cells
Vector Fugene™ (Roche) +CombiMAG. 15 min transfection on MagnetoFACTOR plate
(Data kindly provided by Ch. Plank. Technical University Munich)

4.5.2 CombiMAG - Example Protocols for other Cationic Lipid Reagents

The same steps as for Fugene are carried out. The primary DNA complex is prepared similar to the instructions of the manufacturer.

For example, in step 1, 3.2 µL of Lipofectamine + 96.8 µL medium or 4.0 µL GenePorter + 96 µL medium are used instead of Fugene. The rest of the protocol stays the same.

However, the user is reminded that the alternative mixing order (first mixing DNA and **CombiMAG** followed by mixing with lipid) may be advantageous.

Similarly, the polycationic reagents ExGen500 or Superfect (Qiagen) can be used instead of the lipids.

4.6. Magnetofection™ – Suspension Cells

1. The composition and preparation of **PolyMAG** / DNA or **CombiMAG** / transfection reagent are performed exactly as described above (section 4.4 and 4.5).

2. While **PolyMAG** / DNA or **CombiMAG** / transfection reagent incubate for complex formulation, dilute the cells to be transfected to 5 x 10^5 - 1 x 10^6 / mL in medium (with or without serum- or supplement; depending on cell type and sensitivity of cells towards serum-free conditions) and perform **one of the following three options** to sediment the cells at the bottom of the culture dish in order to promote the contact with the magnetic nanoparticles.

Option 1: Seed the cells on polylysine-coated plates and use the protocol for adherent cells.

Option 2: Briefly, centrifuge the cells (2 minutes) to pellet them and use the protocol for adherent cells.

Option 3: Mix cell suspension with 30 µL of **CombiMAG** reagent per mL of cell suspension.

- Incubate for 10 - 15 minutes.

- Distribute cells to your tissue culture dish placed upon the magnetic plate (volume of culture medium containing cells depends on the culture dish size; see suggested transfection volume in table section 4.2.

- Incubate for 15 minutes

3. Add the resulting complex of **PolyMAG** / DNA or **CombiMAG** / transfection reagent to the cells while keeping the cell culture plate on the **MagnetoFACTOR** plate.

4. Continue to incubate for 15 minutes.

5. Carefully remove the medium supernatant from the cells and replace with fresh complete medium while the culture plate remains positioned on the **MagnetoFACTOR** plate.
Be careful not to aspirate the magnetically sedimented cells.

6. Remove culture plate from **MagnetoFACTOR** plate.

7. Continue to cultivate cells as desired until evaluation of transgene expression.

4.7. Magnetofection™ - siRNA

RNA interference is a powerful technique to shut down gene expression in cells and organisms. This silencing effect constitutes a very helpful tool to study gene function and is a promising approach for new therapeutic treatments. Short RNA duplexes (siRNA: small interfering RNA, shRNA: small hairpin RNA and dsRNA: double strand RNA) are extremely selective by interacting and inducing the degradation of their specific mRNA targets and thereby inhibit the resulting protein production.
PolyMAG introduces the siRNA duplexes in a variety of cells with a very high efficiency leading to exceptional knockdown effects with low doses of siRNA.

The protocol is very straightforward. Please refer to the tables below for specific amount of the respective compounds and transfection volume.

For instance use 2 µL of **PolyMAG** for 20 nM siRNA final concentration in a 6-well plate (transfection volume 2 mL)

Example dilution procedure of siRNA (1 µM stock solution*) and **PolyMAG / CombiMAG** :

Culture vessel	96-well	24-well	12-well	6-well
Dilution serum-free medium	100 µL	100 µL	100 µL	200 µL
Final siRNA concentration	ng siRNA / µL PolyMAG **or** CombiMAG	ng siRNA / µL PolyMAG **or** CombiMAG	ng siRNA / µL PolyMAG **or** CombiMAG	ng siRNA / µL PolyMAG **or** CombiMAG
2 nM	5.4 / 0.5	19.5 / 1.0	27 / 2.0	54 / 2.0
5 nM	19.5 / 0.5	39.75 / 1.0	67.5 / 2.0	135 / 2.0
10 nM	27 / 0.5	67.5 / 1.0	135 / 2.0	270 / 2.0
20 nM	54 / 0.5	135 / 1.0	270 / 2.0	540 / 2.0
50 nM	135 / 0.5	337.5 / 1.0	675 / 2.0	1350 / 2.0
75 nM	202.5 / 1.0	506.25 / 2.0	1012.5 / 2.0	2025 / 2.0

*ng of siRNA was calculated on the basis of a MW = 13 500 g/mol

4.7.1 siRNA – PolyMAG Protocol

1. Plate the cells the day before transfection or just before transfection in your appropriate tissue culture dish and volume of culture medium as suggested (see table section 4.2).

2. Dilute the siRNA to 100 µL (or 200 µL) with culture medium **without** serum and supplement (such as DMEM) (see table for siRNA dilution procedure).

3. Vortex the **PolyMAG** tube before each use. If required, **PolyMAG** can be diluted only with deionized water.
Don't dilute PolyMAG with serum or supplement-free serum.

4. Add directly the appropriate volume/amount of **PolyMAG** to 100 µL (or 200 µL) of the diluted siRNA solution and mix immediately 4 - 5 times by vigorous pipetting.

5. Incubate the formed siRNA / **PolyMAG** complex 15 minutes at room temperature.

6. Add 100 µL (or 200 µL) of the binary complex drop by drop onto the cells.

Note: For some cells, serum-free condition for the first 3 hours of incubation might lead to better gene silencing. However, in most assays, siRNA delivery has been realized in culture medium with serum.

7. Place the cell culture plate upon the **MagnetoFACTOR** plate and incubate under standard cell culture conditions for 10-20 minutes.

8. Remove the **MagnetoFACTOR** plate.

9. Cultivate the cells under standard conditions until evaluation of the gene silencing. Depending on the siRNA amount, the gene target and the cell type assays can be monitored 24 to 96 hours post-transfection.
We recommend 48 hours and 72 hours for RNA and protein knockdown analyses, respectively.

Note: Optionally a medium change can be performed 8-24 h after the transfection if your cells are sensitive to serum/supplement concentration.

4.7.2 siRNA – CombiMAG Protocol

For instance:

• Use 2 µL of **CombiMAG** for 20 nM siRNA final concentration in a 6-well plate (Transfection Volume 2 mL)

1. Plate the cells the day before transfection or just before transfection in your appropriate tissue culture dish and volume of culture medium as suggested (see table section 4.2).

2. Prepare the binary siRNA / Cationic Lipid Reagent complex similar to the instructions of the manufacturer. The final volume should be 100 µl (or 200 µl) with culture medium **without** serum and supplement (such as DMEM) (see table for siRNA dilution procedure).

3. Vortex the **CombiMAG** tube before each use. If required, **CombiMAG** can be diluted only with deionized water.
Don’t dilute CombiMAG with serum or supplement-free serum.

4. Add directly the appropriate volume/amount of **CombiMAG** to 100 µL (or 200 µL) to the siRNA / Cationic Lipid Reagent complex and mix immediately 4 - 5 times by vigorous pipetting.

5. Incubate the formed siRNA / Cationic Lipid Reagent / **CombiMAG** complex 15 minutes at room temperature.

6. Add 100 µL (or 200 µL) of the ternary complex drop by drop onto the cells.

Note: For some cells, serum-free condition for the first 3 hours of incubation might lead to better gene silencing. However, in most assays, siRNA delivery has been realized in culture medium with serum.

7. Place the cell culture plate upon the **MagnetoFACTOR** plate and incubate under standard cell culture conditions for 10-20 minutes.

8. Remove the **MagnetoFACTOR** plate.

9. Cultivate the cells under standard conditions until evaluation of the gene silencing. Depending on the siRNA amount, the gene target and the cell type assays can be monitored 24 to 96 hours post-transfection. We recommend 48 hours and 72 hours for RNA and protein knockdown analyses, respectively.

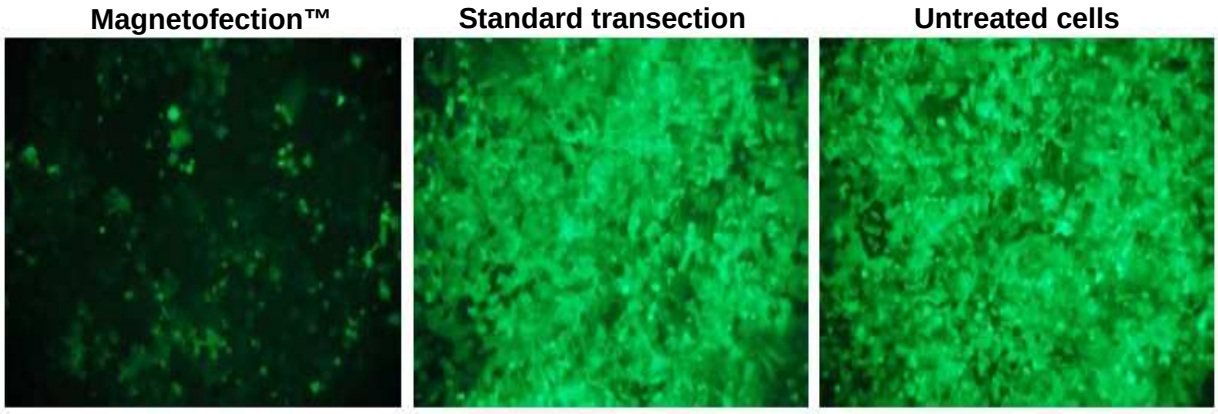
Note: Optionally a medium change can be performed 8-24 h after the transfection if your cells are sensitive to serum / supplement concentration.

Cell culture conditions:

Best results are achieved when cells are 60 - 80 % confluent at the time of the transfection. If necessary, you can wash the culture medium containing the transfection mixture after 8-24 hours and replace it by fresh medium.

siRNA concentration:

We often observed good siRNA effects by concentrations from 10 nM to 50 nM. However the efficiency may depend on the cell line, the target (half life, expression level…) and the siRNA used. Consequently, we suggest to start by testing a range of siRNA concentrations in order to obtain the best experimental conditions.



Magnetofection of siRNA. Synthetic siRNA directed against eGFP was purchased from MWG Biotech, Ebersberg, Germany. The RNA was mixed with PEI-coated magnetic particles and additional free linear PEI (25 kDa, Polysciences, Warrington, PA, USA) in DMEM. After 30 min incubation, the mixture was diluted 51.2-fold with DMEM containing 10% FCS to result in a final siRNA concentration of 65 nM. Aliquots of this mixture (150 µL) were added to HT1080 cells (5000 cells/well in a 96-well plate) which had previously been stably transduced with a retroviral vector coding for eGFP. The culture plate was placed on a magnetic plate during the first 15 min of incubation. A medium change was performed after 24 h. The figure shows the cells 65 h after transfection, documenting an efficient knock down of eGFP expression.
(Data kindly provided by Ch. Plank. Technical University Munich)

4.8. Magnetofection™ - Viruses – CombiMAG Protocol

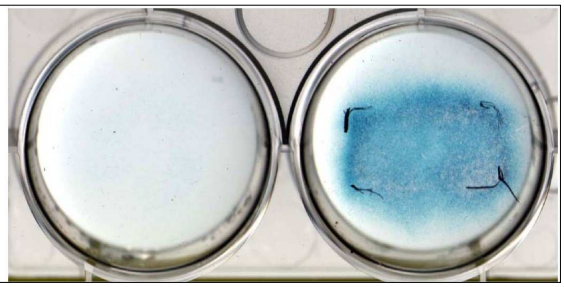
Viral infection is highly cell surface receptor-dependent. For instance, adenoviruses are dependent on cells to express CAR (Coxsackie's and adenovirus receptor) and HIV on cells to express CD4.

Unfortunately, many important and interesting target tissues for fundamental research and gene therapy are non-permissive to viral gene delivery (tumor tissues and apical surface of lung epithelium may express variable, little or none of the required receptors).

• The association of viral vectors with **CombiMAG** is sufficient to force infection of non-permissive cells as shown with adenovirus.

• Magnetofection™ also increases retroviral infectious capacity.

NIH 3T3 cells (lacking the coxsackie and adenovirus receptor, CAR) were transfected with a recombinant adenovirus (coding for LacZ) mixed with CombiMAG in the presence and in the absence of permanent magnets positioned under the culture plates.



1. Cells should be plated in the same manner as required for standard viral gene delivery. For example, the confluency can be high for adenoviral vectors but must be low for retroviral vectors, which require cell division for infection. Cells must be plated the day prior transfection.

2. Provide a suitable amount (see examples below) of **CombiMAG** in a tube large enough to contain the volume of virus preparation added in step 3.

3. Add your virus preparation (e.g. retroviral supernatant or purified adenovirus diluted in HBS, PBS or cell culture medium) to the tube(s) containing **CombiMAG** and mix immediately by pipetting or gentle vortexing. Thereafter, incubate 15 minutes at room temperature.

4. The ratios virus / **CombiMAG** should be adjusted according to the viral titers and cell types used. For optimization, we suggest as a starting point to use 1.5 µL, 3 µL, 6 µL and 12 µL of CombiMAG with a fixed quantity of virus preparation / supernatant.

5. Add the mixture prepared in step 3 to the cells in duplicate or triplicate.

6. Place the cell culture plate upon the magnetic plate and incubate under standard cell culture conditions for 10-20 minutes.

7. Remove the **MagnetoFACTOR** plate. Optionally perform a medium change.

8. Cultivate the cells under standard conditions until evaluation of transgene expression.

9. Depending on the viral vector type, the quantity of virus and the cell types used, this protocol would have to be adjusted.

Cell Type	Virus	CombiMAG
K562	Adenovirus (200 MOI)	6 µL
Human PBL	Adenovirus (500 MOI)	3 – 6 µL
NIH-3T3	Adenovirus (200 MOI)	3 – 6 µL
NIH-3T3	Retrovirus (1-5 x 10^3 X gal CFU/ml)	3 – 6 µL

4.9. High Throughput Optimizations

The ratios of **PolyMAG** or **CombiMAG** to DNA can be varied by doubling or multiplying the volumes of the reagents used.

Similarly, the reagents can be pre-diluted in deionized water and aliquots of the resulting dilutions are incubated with DNA or pre-formed DNA complexes, respectively, such as described above.

Finally, the assembled magnetofectins can be serially diluted to very low concentrations.

4.10. Magnetofection™ - Optimization Protocol in 96-well Format

We recommend to optimize the transfection conditions in order to get the best results of Magnetofection™. Several parameters can be optimized:

• Ratio of **PolyMAG / CombiMAG** to nucleic acid or virus

• Amount of nucleic acid

• Cell density

• Incubation time

For adherent cells, seed the cells at the desired density in a 96-well plate the day prior or at least several hours prior transfection in a total of 150 µL medium per well.

1. In four tubes, dilute 7.2 µg DNA with 352.8 µL **serum- and supplement-free medium** (e.g. DMEM)

2. Add 3.6 µL, 7.2 µL, 10.8 µL and 14.4 µL of **PolyMAG** (in case of DNA) or **CombiMAG** (in case of DNA-transfection reagent complex) reagent in well A1, A4, A7 and A10 of a 96-well plate.

3. Add the 352.8 µL DNA solution from step 1 to well A1, A4, A7, A10 containing **PolyMAG** and mix well by pipetting. Incubate for 15 min at room temperature.

4. **(Optional)** Perform a medium change for the cells to be transfected. Remove medium the cells have been seeded in and replace with 150 µL fresh medium (with or without serum or supplements).

5. In the meantime add 180 µL **serum- and supplement-free medium** (e.g. DMEM) to the residual wells of column 1, 4, 7 and 10 of the 96-well plate (B1-H1, B4-H4, B7-H7, B10-H10).

6. After the incubation in step 3 transfer 180 µL from well A1, A4, A7, A10 to B1, B4, B7, B10, mix by pipetting, transfer 180 µL from B1, B4, B7, B10 to C1, C4, C7, C10, mix by pipetting and so on down to H1, H4, H7, H10.

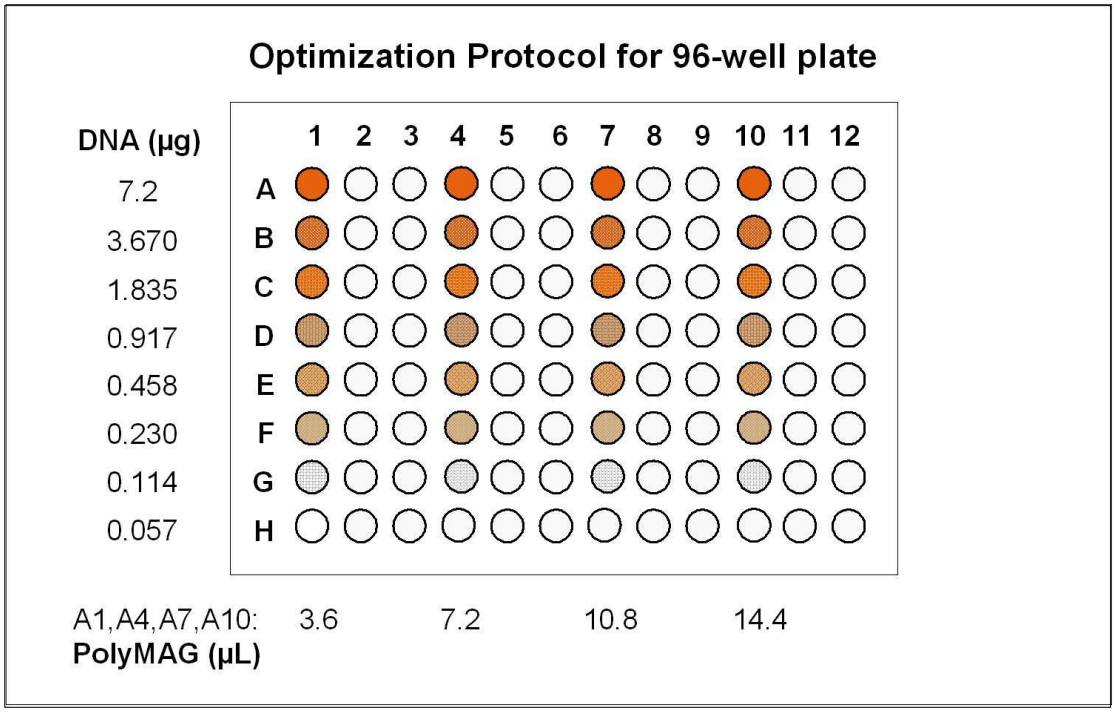
7. Transfer 50 µL each in duplicate from column 1, 4, 7 and 10 to the columns of the cell culture plate where the cells to be transfected have been seeded (column 2/3 , 5/6 , 8/9 , 11/12).

8. Place the culture plate on the **MagnetoFACTOR** plate and incubate under cell culture conditions for 10-20 min.

9. Remove **MagnetoFACTOR** plate.

10. **(Optional)** Perform a medium change, particularly if the transfection has been carried out in **serum-free medium**.

11. Continue to culture cells as desired.



4.11. Troubleshooting

Low Transfection Efficiency

- **Inappropriate buffer composition:**
  Serum-free buffer or medium has to be used for the formation of the PolyMAG/DNA- or CombiMAG/DNA complex, otherwise proteins from the serum will bind to PolyMAG or CombiMAG. Once the PolyMAG/DNA- or CombiMAG/DNA complex is formed it can be applied to cells in the presence of serum.

- **Suboptimal ratio of PolyMAG or CombiMAG to nucleic acid or virus:**
  Determine the optimal ratio of PolyMAG or CombiMAG to DNA by using the optimization protocol for 96-well plate (section 4.10).

- **Correct handling of the MagnetoFACTOR plate:**
  Use the MagnetoFACTOR plate with the magnets facing up. After addition of the PolyMAG/DNA- or CombiMAG/DNA complex to the cells, position the cell culture plate on the MagnetoFACTOR plate.

- **Positive control:**
  Perform a positive control transfection experiment with a well-characterized reporter gene (e,g. GFP, Luciferase).

- **Mycoplasma contamination:**
  Mycoplasma contamination alters transfection efficiency.

- **Cell condition:**
  Cells that have been in culture for a long time may become resistant to transfection. Use freshly thawed cells that have been passaged at least once.

Cellular Toxicity

- **Cell density (% confluence) was not optimal at the time of transfection:**
  Adherent cells are seeded such that they reach 60-80 % confluency at the time of Magnetofection™. If the cell density is too low, increased toxicity may be observed. For suspension cells it is necessary that the cells are immobilized on the well bottom (section 4.6).

- **Suboptimal amount of DNA (section 4.10):**
  Using Magnetofection™, approximately 5 x less DNA compared to Lipofection is necessary.

- **Purity of transfecting molecule:**
  Check the purity of the molecule of interest to be delivered (lipopolysaccharides which are endotoxins will cause cell death).

- **Amounts of PolyMAG or CombiMAG:**
  Higher amounts of PolyMAG or CombiMAG may be cell toxic and can additional reduce the transfection efficiency.

- **Incubation time on the MagnetoFACTOR plate:**
  Longer incubation times (e.g. 4 hours) with PolyMAG/DNA- or CombiMAG/DNA complex plus application of an magnetic field can cause toxic effects.

- **Incubation time after Magnetofection™:**
  Reduce the incubation time of complexes with the cells. Transfection medium can be replaced by fresh medium after 4 hours.

References

9.001 Plank C., Scherer F., Schillinger U., Anton M. **Magnetofection: Enhancement and localization of gene delivery with magnetic particles under influence of a magnetic fields.** J. Gene Med. 2000; 2((5) Suppl.): S24.

9.002 Scherer F., Anton M., Schillinger U., Henke J., Bergemann C., Krüger A., Gänsbacher B. and Plank C. **Magnetofection: enhancing and targeting gene delivery by magnetic force in vitro and in vivo.** Gene Therapy 2002; 9: 102-109.

9.003 Plank C., Schillinger U., Scherer F., Bergemann C., Remy J.-S., Krötz F., Anton M., Lausier J. and Rosenecker J. **The Magnetofection Method: Using Magnetic Force to Enhance Gene Delivery.** Biol. Chem. May 2003; 384: 737-747.

9.004 Plank C., Anton M., Rudolph C., Rosenecker J. and Krötz F. **Enhancing and targeting nucleic acid delivery by magnetic force.** Expert Opinion on Biological Therapy 2003; 3: 745-758.

9.005 Plank C., Scherer F., Schillinger U., Bergemann C., Anton M. **Magnetofection: enhancing and targeting gene delivery with superparamagnetic nanoparticles and magnetic fields.** J. Liposome Res. 2003; 13(1): 29-32.

9.006 Plank C. **Magnetofection: enhancing and targeting gene delivery with lipid-DNA vectors by magnetic force.** J. Liposome Res. 2003; 13(1): 105-106.

9.007 Krötz F., Son HY., Gloe T., Planck C. **Magnetofection Potentiates Gene Delivery to Cultured Endothelial.** Journal of Vascular Research 2003; 40: 425-434.

9.008 Krötz F., Wit C., Sohn HY., Zahler S., Gloe T., Pohl U. and Plank C. **Magnetofection-A highly efficient tool for antisense oligonucleotide delivery in vitro and in vivo.** Mol. Ther. May 2003; 7(5): 700-710.

9.009 Bridell H. **Magnetic field guided nucleic acid delivery to target the Raf-1 kinase in human umbilical vein endothelial cells.** Master thesis in medical science 2003, Technical University of Munich, Germany.

9.010 Gersting S-W., Schillinger U., Lausier J., Nicklaus P., Rudolph C., Plank C., Reinhardt D. and Rosenecker J. **Gene delivery to respiratory epithelial cells by magnetofection.** J.Gene Medicine 2004; 6: 913-922.

9.011 Griesenbach U., Duncan M. Geddes and Eric W.F.W. Alton. **Advances in cystic fibrosis gene therapy**. Current Opinion in Pulmonary Medicine 2004; 10: 542–546.

9.012 Huth S., Lausier J., Gersting S-W., Rudolph C., Plank C., Welsch U. and Rosenecker J. **Insights into the mechanism of magnetofection using PEI-based magnetofectins for gene transfer.** J.Gene Medicine 2004; 6: 923-936.

9.013 Rashmi K. Ambasta, Pravir Kumar et al. **Direct Interaction of the Novel Nox Proteins with p22phox Is required for the Formation of a Functionally Active NADPH Oxidase.** J. of Biological Chem. 2004; 279 (44): 45935-45941.

9.014 Weizhong W., XU Chunfang, WU Hua **Use of PEI-coated Magnetic Iron Oxide Nanoparticles as Gene Vectors** Journal of Huazhong University of Science and Technology (Med. Sci.) 2004; 24 (6): 618-620.

References

9.015 Basile J. R.,Talayeh Afkhami and J. Silvio Gutkind. **Semaphorin 4D/Plexin-B1 Induces Endothelial Cell Migration through the Activation of PYK2, Src, and the Phosphatidylinositol 3-Kinase–Akt Pathway**. Molecular and Cellular Biology 2005; 25 (16): 6889–6898.

9.016 Kadota S., Kanayama T., Miyajima N., Takeuchi K. and Nagata K. **Enhancing of measles virus infection by magnetofection.** J. of Virol. Methods 2005; 128(1-2): 61-66.

9.017 Chudotvorova I., Ivanov A., Rama S., Hübner C.A., Pellegrino C., Ben-Ari Y. and Medina I. **Early expression of KCC2 in rat hippocampal cultures augments expression of functional GABA synapses.** J. Physiol. 2005; 566.3: 671-679.

9.018 Fransen M., Ilse Vastiau, Chantal Brees, Vanessa Brys Guy P. Mannaerts and Paul P. Van Veldhoven. **Analysis of Human Pex19p's Domain Structure by Pentapeptide Scanning Mutagenesis**. J. Mol. Biol. 2005; 346: 1275–1286.

9.019 Goulimari P. et al. **G$\alpha_{12/13}$ Is Essential for Directed Cell Migration and Localized Rho-Dia1 Function.** JBC 2005; 280 (51): 42242-42251.

9.020 Hollander K., Michal Bar-Chen, Shimon Efrat. **Baculovirus p35 increases pancreatic *b*-cell resistance to apoptosis.** Biochemical and Biophysical Research Communications 2005; 332: 550–556.

9.021 Krötz F., Engelbrecht B., Buerkle MA., Bassermann F., Bridell H., Gloe T., Duyster J., Pohl U. and Sohn HY. **The tyrosine phosphatase, SHP-1, is a negative regulator of endothelial superoxide formation.** J. Am. Coll. Cardiol. 2005; 45(10): 1700-1706.

9.022 Recklies A.D., Hua Ling, Chantal White, and Suzanne M. Bernier. **Inflammatory Cytokines Induce Production of CHI3L1 by articular Chondrocytes**. J. of Biol. Chem. 2005; 280 (50): 41213-41221.

9.023 Uchida Y. et al. **Semaphorin 3A signalling is mediated via sequential Cdk5 and GSK3β phosphorylation of CRMP2: implication of common phosphorylating mechanism underlying axon guidance and Alzheimer's disease**. Genes to Cells 2005; 10: 165-179.

9.024 Schillinger et al. **Advances in magnetofection-magnetically guided nucleic acid delivery.** Journal of Magnetism and Magnetic Materials 2005; 293: 501-508.

9.025 Kumbrink J., Gerlinger M. and Johnson J. P. **Egr-1 Induces the Expression of Its Corepressor Nab2 by Activation of the Nab2 Promoter Thereby Establishing a Negative Feedback Loop.** JBC 2005; 280 (52): 42785-42799.

9.026 Liman J. et al. **Interaction of BAG1 and Hsp70 Mediates Neuroprotectivity and Increases Chaprone Activity**. Mol. and Cell. Biology 2005; 25 (9): 3715-3725.

9.027 Stadler S. C., Roman Polanetz, Stephan Meier, Peter U. Mayerhofer, Johannes M. Herrmann, Katja Anslinger, Adelbert A. Roscher, Wulf Röschinger, Andreas Holzinger **Mitochondrial targeting signals and mature peptides of 3-methylcrotonyl-CoA carboxylase**
Biochemical and Biophysical Research Communications 2005; 334:939–946.

9.028 Basile J. R., Rogerio M. Castilho, Vanessa P. Williams and J. Silvio Gutkind. **Semaphorin 4D provides a link between axon guidance processes and tumor-induced angiogenesis**. PNAS 2006; 103 (24): 9017-9022.

References

9.029 Doshida M. et al. **Raloxifene Increases Proliferation and Up-regulates Telomerase Activity in Human Umbilical Vein Endothelial Cells**. The Journal of Biological Chemistry 2006; 281 (34): 24270-24278.

9.030 Kamau Sarah W. et al. **Enhancement of the efficiency of non-viral gene delivery by application of pulsed magnetic field.** Nucleic Acid Research 2006; 34 (5).

9.031 Kaneko M., Weiwen Yang, Yoshiki Matsumoto, Fujiko Watt, Keiko Funa. **Activity of a novel PDGF β-receptor enhancer during the cell cycle and upon differentiation of neuroblastoma**. Experimental Cell Research 2006; 312: 2028-2039.

9.032 Kaur S. et al. **Robo4 Signaling in Endothelial Cells Implies Attraction Guidance Mechanisms**. J. of Biol. Chem. 2006; 281 (16): 11347-11356.

9.033 Kim E-A., Yoon Tae Noh, Myung-Jeom Ryu, Hyun-Taek Kim, Sung-Eun Lee, Cheol-Hee Kim, Cheolju Lee, Young Ho Kim and Cheol Yong Choi. **Phosphorylation and Transactivation of Pax6 by Homeodomain-interacting Protein Kinase 2.** J. of Biolog. Chem. 2006; 281 (11): 7489–7497.

9.034 Nagata D., Takahashi M., Sawai K., Tagami T., Usui T., Shimatsu A., Hirata Y., Naruse M. **Molecular Mechanism of the Inhibitory Effect of Aldosterone on Endothelial NO Synthase Activity.** Hypertension 2006; 48: 165-171.

9.035 Pasder O. et al. **Downregulation of Fer induces PP1 activation and cell-cycle arrest in malignant cells.** Oncogene 2006; 25: 4194-4206.

9.036 Pinto M.P., Grou C.P., Alencastre I.S., Oliveira M.E., Miranda C.S., Fransen M. and Azevedo J.E. **The Import Competence of a Peroxisomal Membrane Protein Is Determined by Pex19p before the Docking Step.** The J. of Biolog. Chem. 2006; 281(45): 34492–34502.

9.037 Romero D. G., Welsh B.L., Gomez-Sanchez E.P., Yanes L.L., Rilli S. and Gomez-Sanchez C.E. **Angiotensin II-Mediated Protein Kinase D Activation Stimulates Aldosterone and Cortisol Secretion in H295R Human Adrenocortical Cells.** Endocrinology 2006; 147(12): 6046–6055.

9.038 Sapet C., Simoncini S., Loriod B., Puthier D., Sampol J., Nguyen C., Dignat-George F. and Anfosso F. **Thrombin-induced endothelial microparticle generation: identification of a novel pathway involving ROCK-II activation by caspase-2.** Blood 2006; 108: 1868-1876.

9.039 Seki T., Irie N., Nakamura K., Sakaue H., Ogawa W., Kasuga M., Yamamoto H., Ohmori S., Saito N. and Sakai N. **Fused protein of δPKC activation loop and PDK1-interacting fragment (δAL-PIF) functions as a pseudosubstrate and an inhibitory molecule for PDK1 when expressed in cells.** Genes to Cells 2006; 11: 1051–1070.

9.040 Wang J., Zhang Y, Wei J., Zhang X., Zhang B., Zhu Z., Zou W., Wang Y., Mou Z., Ni B. and Wu Y. **Lewis X oligosaccharides targeting to DC-SIGN enhanced antigen-specific immune response.** Immunology 2006; 121: 174–182.

9.041 Weizhong W., Xu C., Wu H. **Magnetic Iron Oxide Nanoparticles Mediated Gene Therapy for Breast Cancer－An *In Vitro* Study.** Journal of Huazhong University of Science and Technology (Med. Sci.) 2006; 26 (6): 728-730.

9.042 Xenariou S. et al. **Using magnetic forces to enhance non-viral gene transfer to airway epithelium in vivo.** Gene Therapy 2006; 1-8.

References

9.043 McCAIG C et al. **The Role of Matrix Metalloproteinase-7 in Redefining the astric Microenvironment in Response to Helicobacter pylori.** Gastroenterology 2006; 130:1754–1769.

9.044 Minami R., Yamamoto M., Takahama S., Miyamura T., Watanabe H., Suematsu E. **RCAS1 induced by HIV-Tat is involved in the apoptosis of HIV-1 infected and uninfected CD4+ T cells.** Cellular Immunology 2006; 243: 41–47.

9.045 Mizutani C. et al. **Mechanisms of establishment of persistent SARS-CoV-infected cells**. Biochemical and Biophysical Research Communications 2006; 347: 261-265.

9.046 Mizutani C. et al. **Inhibition of cell proliferation by SARS-CoV infection inVeroE6cells**. FEMS Immunol Med Microbiol 2006; 46: 236-249.

9.047 Mukai H.Y., Motohashi H., Ohneda O., Suzuki N., Nagano M. and Yamamoto M. **Transgene Insertion in Proximity to the c-*myb* Gene Disrupts Erythroid-Megakaryocytic Lineage Bifurcation.** Mol. and Cell. Biology 2006; 26 (21): 7953–7965.

9.048 Rubic T., Reinhard L. Lorenz **Downregulated CD36 and oxLDL uptake and stimulated ABCA1/G1 and cholesterol efflux as anti-atherosclerotic mechanisms of interleukin-10.** Cardiovascular Research 2006; 69: 527 – 535.

9.049 Kojima T., Ken-ichi Nakahama, Kiyotaka Yamamoto, Hiroshi Uematsu and Ikuo Morita **Age- and cell cycle-dependent changes in EPC-1/PEDF promoter activity in human diploid fibroblast-like (HDF) cells.** Molecular and Cellular Biochemistry 2006; 293: 63–69.

9.050 Baer K., Bürli T., Huh K-H, Wiesner A., Erb-Vögtli S., Göckeritz-Dujmovic D., Moransard M., Nishimune A., Rees M.I., Henley J.M., Fritschy J-M and Fuhrera C. **PICK1 interacts with α7 neuronal nicotinic acetylcholine receptors and controls their clustering.** Mol. Cell. Neurosci. 2007; 35: 339–355.

9.051 Basile J.R., Holmbeck K., Bugge T.H. and Gutkind J.S. **MT1-MMP Controls Tumor-induced Angiogenesis through the Release of Semaphorin 4D.** J. of Biolog. Chem. 2007; 282 (9): 6899–6905.

9.052 Buerli T., Pellegrino C., Baer K., Lardi-Studler B., Chudotvorova I., Fritschy J-M., Medina I. and Fuhrer C. **Efficient transfection of DNA or shRNA vectors intoneurons using Magnetofection** NATURE PROTOCOLS 2007; 2(12): 3090-3101.

9.053 Couchoux H., Allard B., Legrand C., Jacquemond V. and Berthier C. **Loss of caveolin-3 induced by the dystrophy-associated P104L mutation impairs L-type calcium channel function in mouse skeletal muscle cells.** J. of Physiol. 2007; 580.3: 745–754.

9.054 Deleuze V., Chalhoub E., El-Hajj R., Dohet C., Le Clech M., Couraud P-O., Philippe H. and Mathieu D. **TAL-1/SCL and Its Partners E47 and LMO2 Up-Regulate VE-Cadherin Expression in Endothelial Cells.** Mol. and Cell. Biology 2007; 27 (7): 2687–2697.

9.055 Eder P., D. Probst, C. Rosker, M. Poteser, H. Wolinski, S.D. Kohlwein, C. Romanin, K. Groschner **Phospholipase C-dependent control of cardiac calcium homeostasis involves a TRPC3-NCX1 signaling complex.** Cardiovascular Research 2007; 73: 111–119.

References

9.056 Fukushima T., Tezuka T., Shimomura T., Nakano S. and Kataoka H. **Silencing of Insulin-like Growth Factor-binding Protein-2 in Human Glioblastoma Cells Reduces Both Invasiveness and Expression of Progression-associated Gene CD24.** J. of Biolog. Chem. 2007; 282 (25): 18634–18644.

9.057 Jahnke A., Hirschberger J., Fischer C., Brill T., Köstlin R., Plank C., Küchenhoff H., Krieger S., Kamenica K. and Schillinger U. **Intra-tumoral Gene Delivery of feIL-2, feIFN-c and feGM-CSF using Magnetofection as a Neoadjuvant Treatment Option for Feline Fibrosarcomas: A Phase-I Study.** J. Vet. Med. A 2007; 54: 1-8.

9.058 Romero D.G., Yanes L.L., de Rodriguez A.F., Plonczynski M.W., Welsh B.L., Reckelhoff J.F., Gomez-Sanchez E.P. and Gomez-Sanchez C.E. **Disabled-2 (Dab2) is Expressed in Adrenal Zona Glomerulosa and is Involved in Aldosterone Secretion.** Endocrinology 2007, First published ahead of print February 15, 2007 as doi:10.1210/en. 2006-1509.

9.059 Romero D.G., Rilli S., Plonczynski M.W., Yanes L.L., Zhou M.Y., Gomez-Sanchez E.P. and Gomez-Sanchez C.E. **Adrenal transcription regulatory genes modulated by angiotensin II and their role in steroidogenesis.** Physiol. Genomics 2007; 30: 26–34.

9.060 Sacha J.B., Chung C., Rakasz E.G., Spencer S.P., Jonas A.K., Bean A.T., Lee W., Burwitz B.J., Stephany J.J., Loffredo J.T, Allison D.B., Adnan S., Hoji A., Wilson N.A., Friedrich T.C., Lifson J.D., Yang O.O. and Watkins D.I. **Gag-Specific $CD8^+$ T Lymphocytes Recognize Infected Cells before AIDS-Virus Integration and Viral Protein Expression.** The Journal of Immunology 2007; 178: 2746–2754.

9.061 Steele I.A., Rod Dimaline R., Pritchard D.M., Peek Jr. R.M., Wang T.C., Dockray G.J. and Varro A. ***Helicobacter* and gastrin stimulate Reg1 expression in gastric epithelial cells through distinct promoter elements.** Am. J. Physiol. Gastrointest. Liver Physiol. 2007; 293: G347–G354.

9.062 Schäfer C., Hoffmann L., Heldt K., Reza M., Lornejad-Schäfer, Brauers G., Gehrmann T., Garrow T.A., Häussinger D., Mayatepek E., Schwahn B.C. and Schliess F. **Osmotic regulation of betaine homocysteine-S-methyltransferase expression in H4IIE rat hepatoma cells.** Am J Physiol Gastrointest Liver Physiol 2007; 292:1089-1098.

9.063 Tan Z., X Sun, F-S Hou, H-W Oh, LGW Hilgenberg, EM Hol, FW van Leeuwen, MA Smith, DK O'Dowd and SS Schreiber. **Mutant ubiquitin found in Alzheimer's disease causes neuritic beading of mitochondria in association with neuronal degeneration.** Cell Death and Differentiation 2007; 14: 1721–1732.

9.064 Nakamura A., Yukiko Sakai, Chieri Ohata, Toshi Komurasaki. **Expression and significance of a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)-1 in an animal model of renal interstitial fibrosis induced by unilateral ureteral obstruction.** Experimental and Toxicologic Pathology 2007; 59: 1–7.

9.065 McBain S.C., H. H. P. Yiu, A. El Haj and J. Dobson. **Polyethyleneimine functionalized iron oxide nanoparticles as agents for DNA delivery and transfection.** Journal of Materials Chemistry 2007; 17: 2561–2565.

9.066 Huang P., T Senga and M Hamaguchi. **A novel role of phospho-b-catenin in microtubule regrowth at centrosome.** Oncogene 2007; 26: 4357–4371.

9.067 Hasegawa T., Toshiyuki Matsuzaki, Yuki Tajika, Abduxukur Ablimit, Takeshi Suzuki, Takeo Aoki Haruo Hagiwara, Kuniaki Takata. **Differential localization of aquaporin-2 and glucose transporter 4 in polarized MDCK cells.** Histochem Cell Biol 2007; 127: 233–241.

References

9.068 Guix S., Miyuki Asanaka, Kazuhiko Katayama, Sue E. Crawford, Frederick H. Neill, Robert L. Atmar, and Mary K. Estes. **Norwalk Virus RNA Is Infectious in Mammalian Cells.** Journal of Virology 2007; 81(22): 12238 – 12248.

9.069 Varro A., Kenny S., Hemers E., McCaig C., Przemeck S., Wang T.C., Bodger K. and Pritchard D.M. **Increased gastric expression of MMP-7 in hypergastrinemia and significance for epithelial-mesenchymal signalling.** Am. J. Physiol. Gastrointest. Liver Physiol. 2007; 292: G1133–G1140.

9.070 Lardi-Studler B., Smolinsky B., Petitjean C.M., Koenig F., Sidler C., Meier J.C., Fritschyl J-M. and Schwarz G. **Vertebrate-specific sequences in the gephyrin E-domain regulate cytosolic aggregation and postsynaptic clustering.** Journal of Cell Science 2007; 120: 1371-1382.

9.071 Lartigue G., Dimaline R., Varro A. and Dockray G.J. **Cocaine- and Amphetamine-Regulated Transcript: Stimulation of Expression in Rat Vagal Afferent Neurons by Cholecystokinin and Suppression by Ghrelin.** The Journal of Neuroscience 2007; 27(11): 2876 –2882.

9.072 Mykhaylyk O., Vlaskoua D., Tresilwised N., Pithayanukul P, Möller W., Plank C. **Magnetic nanoparticle formulations for DNA and siRNA delivery.** J.of Magnetism and Magnetic Materials 2007; 311: 275–281.

9.073 Mykhaylyk O., Antequera Y.S., Vlaskou D. & Plank Ch. **Generation of magnetic nonviral gene transfer agents and Magnetofection in vitro.** Nature Protocols 2007; 2(10): 1-21.

9.074 Yim H-J., Kim H-J., Ko J-H., Cho Y-E., Kim S-M., Kim J-Y., Lee S. and Park J-H. **The putative tumor suppressor gene GLTSCR2 induces PTEN-modulated cell death.** Cell Death and Differentiation 2007; 1-8.

9.075 Yim Ji-Hye, Yong-Jun Kim, Young-Eun Cho, Jeong-Hun Ko, Su-Mi Kim, Jee-Youn Kim, Jae-Hoon Park. **GLTSCR2 Sensitizes Cells to Hypoxic Injury without Involvement of Mitochondrial Apoptotic Cascades** Pathobiology 2007;74:301–308.

9.076 Yoon W. S., Won Cheol Choi, Jeong-Im Sin, Yong Keun Park **Antitumor therapeutic effects of Salmonella typhimurium containing Flt3 Ligand expression plasmids in melanoma-bearing mouse.** Biotechnology Letters 2007; 29:511–516.

9.077 Thomas J.A., Ott D.E. and Gorelick R.J. **Efficiency of Human Immunodeficiency Virus Type 1 Postentry Infection Processes: Evidence against Disproportionate Numbers of Defective Virions.** Journal of Virology 2007; 81 (8): 4367–4370.

9.078 Arnoux V., Mayssaa Nassour, Annie L'Helgoualch, Robert A. Hipskind and Pierre Savagner. **Erk5 Controls Slug Expression and Keratinocyte Activation during Wound Healing.** Molecular Biology of the Cell 2008; 19: 4738–4749.

9.079 Ashurst H.L., Andrea Varro and Rod Dimaline. **Regulation of mammalian gastrin/CCK receptor (CCK2R) expression *in vitro* and *in vivo.*** Experimental Physiology 2008; 99.2: 223–236.

9.080 Bataille A.M., James Goldmeyer, and J. Larry Renfro. **Avian renal proximal tubule epithelium urate secretion is mediated by Mrp4.** Am J Physiol Regul Integr Comp Physiol 2008; 295: R2024–R2039.

References

9.081 Burdyga G., Guillaume de Lartigue, Helen E. Raybould, Richard Morris, Rod Dimaline, Andrea Varro, David G. Thompson and Graham J. Dockray. **Cholecystokinin Regulates Expression of Y2 Receptors in Vagal Afferent Neurons Serving the Stomach.** The Journal of Neuroscience 2008; 28(45):11583–11592.

9.082 Cestéle S., Paolo Scalmani, Raffaella Rusconi, Benedetta Terragni, Silvana Franceschetti and Massimo Mantegazza. **Self-Limited Hyperexcitability: Functional Effect of a Familial Hemiplegic Migraine Mutation of the Nav1.1 (SCN1A) Na^+ Channel.** The Journal of Neuroscience 2008; 28(29): 7273–7289.

9.083 Dobson J., SC McBain, N Farrow, CD Batich. **Oscillating magnet arrays for enhanced magnetic nanoparticle-based gene transfection.** European Cells and Materials 2008; 16 Suppl. 3: (page 48).

9.084 Choi S. M., Cho H-J., Cho H., Kim K. H., Kim J. B. and Park H. **Stra13/DEC1 and DEC2 inhibit sterol regulatory element binding protein-1c in a hypoxia-inducible factor-dependet mechanism.** Nucleic Acids Research 2008; Published October 5: 1-14.

9.085 Francois M., Andrea Caprini, Brett Hosking, Fabrizio Orsenigo, Dagmar Wilhelm, Catherine Browne, Karri Paavonen, Tara Karnezis, Ramin Shayan, Meredith Downes, Tara Davidson, Desmond Tutt, Kathryn S. E. Cheah, Steven A. Stacker, George E. O. Muscat, Marc G. Achen, Elisabetta Dejana & Peter Koopman. **Sox18 induces development of the lymphatic vasculature in mice** NATURE 2008; 456: 643-648.

9.086 Guillaud L., Wong R. and Hirokawa N. **Disruption of KIF17–Mint1 interaction by CaMKIIdependent phosphorylation: a molecular model of kinesin–cargo release.** Nature Cell Biology 2008; 10 (1): 19-29.

9.087 Hüttinger C., Hirschberger J., Jahnke A., Köstlin R., Brill T., Plank C., Küchenhoff H., Krieger S. and Schillinger U. **Neoadjuvant gene delivery of feline granulocyte-macrophage colony-stimulating factor using magnetofection for the treatment of feline fibrosarcomas: a phase I trial.** J. of Gene Med. 2008; 10: 655–667.

9.088 Kim Y.-B., Suyong Choi, Moon-Chang Choi, Min-A Oh, Sin-Ae Lee, Moonjae Choy, Kensaku Mizuno, Sung-Hoon Kim and Jung Weon Lee. **Cell Adhesion-dependent Cofilin Serine 3 Phosphorylation by the Integrin-linked Kinase c-Src Complex.** J. of Biolog. Chem. 2008; 283 (15): 10089–10096.

9.089 Kojima A., Ken-ichi Nakahama, Kyoko Ohno-Matsui, Noriaki Shimada, Keisuke Mori, Sachiko Iseki, Tetsuji Sato, Manabu Mochizuki, Ikuo Morita. **Connexin 43 contributes to differentiation of retinal pigment epithelial cells via cyclic AMP signalling.** Biochemical and Biophysical Research Communications 2008; 366: 532–538.

9.090 Kuczewski N., Christophe Porcher, Nadine Ferrand, Hervé Fiorentino, Christophe Pellegrino, Richard Kolarow, Volkmar Lessmann, Igor Medina and Jean-Luc Gaiarsa. **Backpropagating Action Potentials Trigger Dendritic Release of BDNF during Spontaneous Network Activity.** The Journal of Neuroscience 2008; 28(27): 7013–7029.

9.091 Lee C. H., Kim E. J., Jeon K., Tae J. C., Lee K. S., Kim Y. O., Jeong M.-Y., C.-W. Yun et. al. **Simple, Efficient, and Reproducible Gene Transfection of Mouse Embryonic Stem Cells by Magnetofection.** Stem Cells and Development 2008; 17:133–141.

References

9.092 Mannell H., Nicole Hellwig, Torsten Gloe, Christian Plank, Hae-Young Sohn, Leopold Groesser, Barbara Walzog, Ulrich Pohl, Florian Krötz. **Inhibition of the Tyrosine Phosphatase SHP-2 Suppresses Angiogenesis in vitro and in vivo.** Journal of Vascular Research 2008; 45: 153–169.

9.093 Markova O., Marat Mukhtarov, Eleonore Real, Yves Jacob, Piotr Bregestovski. **Genetically encoded chloride indicator with improved sensitivity.** Journal of Neuroscience Methods 2008; 170: 67–76.

9.094 Matchkov V.V., Per Larsen, Elena V. Bouzinova, Aleksandra Rojek, Donna M. Briggs Boedtkjer, Veronika Golubinskaya, Finn Skou Pedersen, Christian Aalkjær and Holger Nilsson. **Bestrophin-3 (Vitelliform Macular Dystrophy 2–Like 3 Protein) Is Essential for the cGMP-Dependent Calcium-Activated Chloride Conductance in Vascular Smooth Muscle Cells.** *Circ. Res.* 2008;103: 864-872.

9.095 McBain S.C., U. Griesenbach, S. Xenariou, A. Keramane, C.D. Batich, E.W.F.W. Alton and J. Dobson. **Magnetic nanoparticles as gene delivery agents: enhanced transfection in the presence of oscillating magnet arrays.** Nanotechnology 2008; 19: 405102 (5pp).

9.096 Mukhtarov M., Markova O., Real E., Jacob Y., Buldakova S., Bregestovski P. **Monitoring of chloride and activity of glycine receptor channels using genetically encoded fluorescent sensors.** Phil. Trans. R. Soc. A 2008; 366: 3445–3462.

9.097 Sbai O., Lotfi Ferhat, Anne Bernard, Yatma Gueye, Adlane Ould-Yahoui, Sophie Thiolloy, Eliane Charrat, Gérard Charton, Evelyne Tremblay, Jean-Jacques Risso, Jean-Paul Chauvin, Jean-Pierre Arsanto, Santiago Rivera, Michel Khrestchatisky. **Vesicular trafficking and secretion of matrix metalloproteinases-2, -9 and tissue inhibitor of metalloproteinases-1 in neuronal cells**. Molecular and Cellular Neuroscience 2008; 39: 549–568.

9.098 Ufer C., Chi Chiu Wang, Michael Fähling, Heike Schiebel, Bernd J. Thiele, E. Ellen Billett, Hartmut Kuhn and Astrid Borchert. **Translational regulation of glutathione peroxidase 4 expression through guaninerich sequence-binding factor 1 is essential for embryonic brain development.** Genes & Development 2008; 22: 1838-1850.

9.099 Yamaguchi T., Yoshihisa Watanabe, Masaki Tanaka, Masanori Nakagawa and Nozomi Yamaguchi. **cAMP-Dependent Regulation of Spinesin/TMPRSS5 Gene Expression in Astrocytes.** Journal of Neuroscience Research 2008; 86: 610–617.

9.100 Yang Shieh-Yueh, Jui-Sheng Sun, Cheng-Heng Liu, Yang-Hwei Tsuang, Li-Ting Chen, Chin-Yih Hong, Hong-Chang Yang, and Herng-Er Horng. **Ex Vivo MagnetofectionWith Magnetic Nanoparticles: A Novel Platform for Nonviral Tissue Engineering.**
Artif Organs 2008; 32 (3):195–204.

9.101 Han S.J., Duong H.-Q., Choi J. E., Lee T.-B., Kim C. H., Lee S. Y., Jeon H. M., Shin S.-H., Lim S.-C. and Kang H. S. **Hyperthermia switches glucose depletion-induced necrosis to apoptosis in A549 lung adenocarcinoma cells.** INTERNATIONAL JOURNAL OF ONCOLOGY 2008; 32: 851-860.

9.102 Mykhaylyk O., Olivier Zelphati, Joseph Rosenecker & Christian Plank **siRNA delivery by magnetofection.** Current Opinion in Molecular Therapeutics 2008 10(5): 1-19.

9.103 Rhee Dong Kuen, Su Hyung Park, Yeun Kyu Jang **Molecular signatures associated with transformation and progression to breast cancer in the isogenic MCF10 model.** Genomics 2008; 92: 419–428.

References

9.104 Cho Li-La, Young-Mee Moon, Yu-Jung Heo, Yun-JuWoo, Ji-Hyeon Ju, Kyung-Su Park, Sung-Il Kim, Sung-Hwan Park, Ho-Youn Kim, Jun-Ki Min. **NF-κB inhibition leads to increased synthesis and secretion of MIF in human CD4+ T cells.** Immunology Letters 2009; 123: 21–30.

9.105 Egaňa L.A., Rolando A. Cuevas, Tracy B. Baust, Leonardo A. Parra, Rehana K. Leak, Sarah Hochendoner, Karina Peňa, Marisol Quiroz, Weimin C. Hong, Mario M. Dorostkar, Roger Janz, Harald H. Sitte and Gonzalo E. Torres. **Physical and Functional Interaction between the Dopamine Transporter and the Synaptic Vesicle Protein Synaptogyrin-9.** The Journal of Neuroscience 2009; 29(14): 4592– 4604.

9.106 Fiorentino H., Nicola Kuczewski, Diabe Diabira, Nadine Ferrand, Menelas N. Pangalos, Christophe Porcher and Jean-Luc Gaiarsa. **$GABA_B$ Receptor Activation Triggers BDNF Release and Promotes the Maturation of GABAergic Synapses.** The Journal of Neuroscience 2009; 29(37): 11650 –11661.

9.107 Ivanov A., Monique Esclapez, Christophe Pellegrino, Tomoaki Shirao and Lotfi Ferhat. **Drebrin A regulates dendritic spine plasticity and synaptic function in mature cultured hippocampal neurons.** Journal of Cell Science 2009; 122: 524-534.

9.108 Kenny S., Cedric Duval, Stephen J. Sammut, Islay Steele, D. Mark Pritchard, John C.Atherton, Richard H. Argent, Rod Dimaline, Graham J. Dockray and Andrea Varro. **Increased expression of the urokinase plasminogen activator system by *Helicobacter pylori* in gastric epithelial cells.** Am J Physiol Gastrointest Liver Physiol. 2009; Article in press.

9.109 Megias J., Maria Isabel Guillen, Victoria Clerigues, Ana I. Rojo, Antonio Cuadrado, Miguel Angel Castejon, Francisco Gomar, Maria Jose Alcaraz. **Heme oxygenase-1 induction modulates microsomal prostaglandin E synthase-1 expression and prostaglandin E2 production in osteoarthritic chondrocytes.** Biochemical Pharmacology 2009; 77: 1806–1819.

9.110 Tanaka M., Yoshihisa Watanabe and Kanji Yoshimoto. **Regulation of Relaxin 3 Gene Expression Via cAMP-PKA in a Neuroblastoma Cell Line.** Journal of Neuroscience Research 2009; 87: 820–829.

9.111 Svingen T., Wilhelm D., Combes A. N., Hosking B., Harley V. R., Sinclair A. H. and Koopman P. **Ex Vivo Magnetofection: A Novel Strategy for the Study of Gene Function in Mouse Organogenesis.** DEVELOPMENTAL DYNAMICS 2009; 238:956–964.

9.112 Wong J. and Cynthia Shannon Weickert **Transcriptional Interaction of an Estrogen Receptor Splice Variant and ErbB4 Suggests Convergence in Gene Susceptibility Pathways in Schizophrenia** THE JOURNAL OF BIOLOGICAL CHEMISTRY 2009; 284(28):18824–18832.

9.113 Yiu H.P., Stuart C. McBain, Zoe A. D. Lethbridge, Martin R. Lees, Jon Dobson. **Preparation and characterization of polyethylenimine-coated Fe3O4-MCM-48 nanocomposite particles as a novel agent for magnet-assisted transfection.** Journal of Biomedical Materials Research Part A 2009; 386-392.

9.114 Hofmann A., Wenzel D., Becher U.M., Freitag D.F., Klein A. M., Eberbeck D., Schulte M., Zimmermann K., Bergemann C., Gleich B., Roell W., Weyh T., Trahms L., Nickenig G., Fleischmann B. K. and Pfeifer A. **Combined targeting of lentiviral vectors and positioning of transduced cells by magnetic nanoparticles.** PNAS 2009; 106 (1): 44-49.

9.115 Hofmann et al. **Supporting Information.** 10.1073/pnas.0803746106, PNAS 2009: 1-9.

References

9.116 Saeki Yuko, Takaho Endo, Kaori Ide, Takeshi Nagashima, NorikoYumoto, TetsuroToyoda, Harukazu Suzuki, Yoshihide Hayashizaki, Yoshiyuki Sakaki and Mariko Okada-Hatakeyama. **Ligand-specific sequential regulation of transcription factors for differentiation of MCF-7 cells.** BMC Genomics 2009; 10: 545.

9.117 Lee K Y, Hyungu Kang, Sung Ho Ryu, Dong Soo Lee, Jung Hwan Lee and Soon hag Kim. **Bioimaging of Nucleolin Aptamer-Containing 5-(N-benzylcarboxyamide)-2'-deoxyuridine More Capable of Specific Binding to Targets in Cancer Cells.** Journal of Biomedicine and Biotechnology 2010; Volume2010, Article ID168306, 9 pages.

9.118 Song Hai Peng, Jing Ye Yang, Seong Loong Lo, Yi Wang, Wei Min Fan, Xiao Sheng Tang, Jun Min Xue, Shu Wang. **Genetransfer using self-assembled ternary complexes of cationic magnetic nanoparticles, plasmid DNA and cell-penetrating Tat peptide.** Biomaterials 2010; 31: 769–778.

9.119 Nakakuki Takashi, Marc R. Birtwistle, Yuko Saeki, NorikoYumoto, Kaori Ide, Takeshi Nagashima, Lutz Brusch, Babatunde A.Ogunnaike, Mariko Okada-Hatakeyama and Boris N. Kholodenko. **Ligand-Specific c-Fos Expression Emerges from the Spatiotemporal Control of ErbB Network Dynamics.** Cell 2010; 141: 1-19.

9.120 Ensenauer R., D. Hartl , J. Vockley, AA. Roscher, U. Fuchs. **Efficient and gentle siRNA delivery by Magnetofection.** Biotechnic & Histochemistry 2010, Early Online, 1–6.

9.121 Holzbach T., Dialekti Vlaskou, Iva Neshkova, Moritz A. Konerding, Klaus Wörtler, Olga Mykhaylyk, Bernd Gänsbacher, H.-G. Machens, Christian Plank, Riccardo E. Giunta. **Non-viral $VEGF_{165}$ gene therapy – magnetofection of acoustically active magnetic lipospheres ('magnetobubbles') increases tissue survival in an oversized skin flap model.** J. Cell. Mol. Med. 2010; Vol 14(3): 587-599.

9.122 Vlaskou D, Olga Mykhaylyk, Florian Krötz, Nicole Hellwig, Ritta Renner, Ulrike Schillinger, Bernhard Gleich, Alexandra Heidsieck, Georg Schmitz, Karin Hensel and Christian Plank. **Magnetic and Acoustically Active Lipospheres for Magnetically Targeted Nucleic Acid Delivery.** Advanced Functional Materials Volume 20, Issue 22, pages 3881–3894, November 23, 2010.

9.123 Pino P., Almudena Munoz-Javier, Dialechti Vlaskou, Pilar Rivera Gil, Christian Plank and Wolfgang J. Parak **Gene Silencing Mediated by Magnetic Lipospheres Tagged with Small Interfering RNA.** Nano Lett., 2010, 10 (10), pp 3914–3921.

9.124 Pino P., Almudena Munoz-Javier, Dialechti Vlaskou, Pilar Rivera Gil, Christian Plank and Wolfgang J. Parak **Gene Silencing Mediated by Magnetic Lipospheres Tagged with siRNA.** Nano Lett., 2010, SUPPORTING INFORMATION.

9.125 Pino P., Almudena Munoz-Javier, Dialechti Vlaskou, Pilar Rivera Gil, Christian Plank and Wolfgang J. Parak. **Gene Silencing Mediated by Magnetic Lipospheres Tagged with siRNA.** Nano Lett., 2010, SUPPORTING INFORMATION.

9.126 Fouriki A., N. Farrow, M.A. Clements and J. Dobson. **Evaluation of the magnetic field requirements for nanomagnetic gene transfection.** Citation: Nano Reviews 2010, 1: 5167 - DOI: 10.3402/nano.v1i0.5167.

9.127 Ang D., Q.V. Nguyen, S. Kayal, P.R. Preiser, R.S. Rawat, R.V. Ramanujan. **Insights into the mechanism of magnetic particle assisted gene delivery.** Acta Biomaterialia 2011; Vol. 7: 1319–1326.

References

9.128 Li Fang, Sumei Niu, Jing Sun, Huaishi Zhu, Qiujie Ba, Yi Guo, and Donglu Shi. **Efficient In Vitro TRAIL-Gene Delivery in Drug-Resistant A2780/DDP Ovarian Cancer Cell Line via Magnetofection.** Journal of Nanomaterials 2011, Article ID 484589, 7 pages.

9.129 Lee Soondong, Gayong Shim, Sunil Kim, Young Bong Kim, Chan-Wha Kim, Youngro Byun, and Yu-Kyoung Oh. **Enhanced Transfection Rates of Small-Interfering RNA Using Dioleylglutamide-Based Magnetic Lipoplexes.** NUCLEIC ACID THERAPEUTICS 2011; Volume 21, Number 3.

9.130 Plank Ch., Olivier Zelphati, Olga Mykhaylyk. Magnetically enhanced nucleic acid delivery. **Ten years of magnetofection — Progress and prospects.** Advanced Drug Delivery Reviews 2011; Article in press, 32 pages.

9.131 Baryshev M., D.Vainauska, S. Kozireva and A.Karpovs. **New Device for Enhancement of Liposomal Magnetofection Efficiency of Cancer Cells.** World Academy of Science, Engineering and Technology 2011; 58: 306-309.

9.132 Naruo Yoshimi, Takeshi Nagashima, Ryoko Ushikoshi-Nakayama, Yuko Saeki, Takashi Nakakuki, Takashi Naka, Hiroshi Tanaka, Shih-Feng Tsai, Mariko Okada-Hatakeyama. **Epidermal growth factor receptor mutation in combination with expression of MIG6 alters gefitinib sensitivity.** BMC Systems Biology 2011, 5:29.

9.133 Cho Ssang-Goo, Sachin Honguntikar and Hyun Joo Lee. **Application of Magnet-based Nanofection in Embryonic Stem Cell Research. Methodological Advances in the Culture,** Manipulation and Utilization of Embryonic Stem Cells for Basic and Practical Applications 2011 Edited by Prof. Craig Atwood (Hard Cover Book) pp 307-327.

9.134 Cui Jinhui, Haixin Cui, YanWang, Changjiao Sun, Kui Li, Hongyan Ren, and Wei Du. **Application of PEI-ModifiedMagnetic Nanoparticles as Gene Transfer Vector for the GeneticModification of Animals.** Advances in Materials Science and Engineering 2011; Vol. 2012, Article ID 764521, 6 pages.

9.135 Durdik S., M. Babincov´a, C. Bergemann and P. Babinec. **Nanosecond laser pulse induced stress waves enhanced magnetofection of human carcinoma cells in vitro.** Laser Phys. 2012 Lett. 9, No. 9, 678–681.

9.136 Vainauska Dace, Svetlana Kozireva, Andrejs Karpovs, Maksims Čistjakovs, Mihails Bariševs. **A Novel Approach for Nucleic Acid Delivery Into Cancer Cells.** Medicina (Kaunas) 2012;48(6):324-9.

9.137 Jeon K, Lim H, Kim JH, Thuan NV, Park SH, Lim YM, Choi HY, Lee ER, Kim JH, Lee MS, Cho SG. **Differentiation and transplantation of functional pancreatic Beta cells generated from induced pluripotent stem cells derived from a type 1 diabetes mouse model.** Stem Cells Dev. 2012 Sep 20;21(14):2642-55. Epub 2012 Jun 1.

9.138 Park Young-Kwon and Hyunsung Park. **Differentiated embryo chondrocyte 1 (DEC1) represses PPARɣ2 gene through interacting with CCAAT/enhancer binding protein ß (C/EBPß).** Mol. Cells 2012; 33: 575-581.

9.139 Saeki Y, Nagashima T, Kimura S, Okada-Hatakeyama M. **An ErbB receptor-mediated AP-1 regulatory network is modulated by STAT3 and c-MYC during calcium-dependent keratinocyte differentiation.** Exp Dermatol. 2012 Apr; 21(4): 293-298.

References

9.140 Sun SL, Lo YL, Chen HY, Wang LF. **Hybrid polyethylenimine and polyacrylic acid-bound iron oxide as a magnetoplex for gene delivery.** Langmuir. 2012 Feb 21; 28(7): 3542-3552.

9.141 Park Hyo Young, Eun Hyung Noh, Hyung-Min Chung, Man-Jong Kang, Eun Young Kim, Se Pill Park. **Efficient Generation of Virus-Free iPS Cells Using Liposomal Magnetofection.** PLOS ONE 2012, Vol.7, Issue 9.

9.142 Prijic Sara , Lara Prosen, Maja Cemazar, Janez Scancar, Rok Romih, Jaka Lavrencak, Vladimir B. Bregar, Andrej Coer, Mojca Krzan, Andrej Znidarsic, Gregor Sersa. **Surface modified magnetic nanoparticles for immuno-gene therapy of murine mammary adenocarcinoma.** Biomaterials 2012; 33: 4379-4391.

9.143 Tencomnao Tewin, Kewalin Klangthong, Nuttaporn Pimpha, Saowaluk, Chaleawlert-umpon Somsak Saesoo, Noppawan, Woramongkolchai, Nattika Saengkrit. **Acceleration of gene transfection efficiency in neuroblastoma cells through polyethyleneimine/poly(methyl methacrylate) core-shell magnetic nanoparticles.** International Journal of Nanomedicine 2012:7 2783–2792.

9.144 Avotina Dace, Svetlana Kozireva, Andrejs Karpovs, Maksims Chistyakovs, Mikhail Baryshev. **Dynamic Magnetic Field Increases Intracellular Magnetic Labelling of Prostate Carcinoma and Burkitt's Lymphoma Cells.** Collection of Scientific Papers: Research articles in medicine & pharmacy, 2012: Internal Medicine. Surgery, pages: 65 – 70.

9.145 Park Youn Hee, Young Mi Lee, Dong Sun Kim, Jaechan Park, Kyoungho Suk, Jong Kun Kim and Hyung Soo Han. **Hypothermia enhances induction of protective protein metallothionein under ischemia.** Journal of Neuroinflammation 2013, 10:21.

9.146 Aurora Sandeep, Girish Gupta, Sukhbir Singh and Neelam Singh. **Advances in Magnetofection – Magnetically Guided Nucleic Acid Delivery: a Review.** Journal of Pharmaceutical Technology, Research and Management May 2013, Vol. 1: pp. 19–29.

9.147 Lee Jung-Tae, Jae-Whan Jung, Jae-Yong Choi, Tae-Geon Kwon. **Enhanced bone morphogenic protein adenoviral gene delivery to bone marrow stromal cells using magnetic nanoparticle.** J Korean Assoc Oral Maxillofac Surg 2013, 39:112-119.

9.148 Wong Jenny, Carlotta E. Duncan, Natalie J. Beveridge, Maree J. Webster, Murray J. Cairns, and Cynthia Shannon Weickert. **Expression of NPAS3 in the Human Cortex and Evidence of Its Posttranscriptional Regulation by miR-17 During Development, With Implications for Schizophrenia.** Schizophrenia Bulletin 2013; vol. 39 no. 2 pp. 396–406.