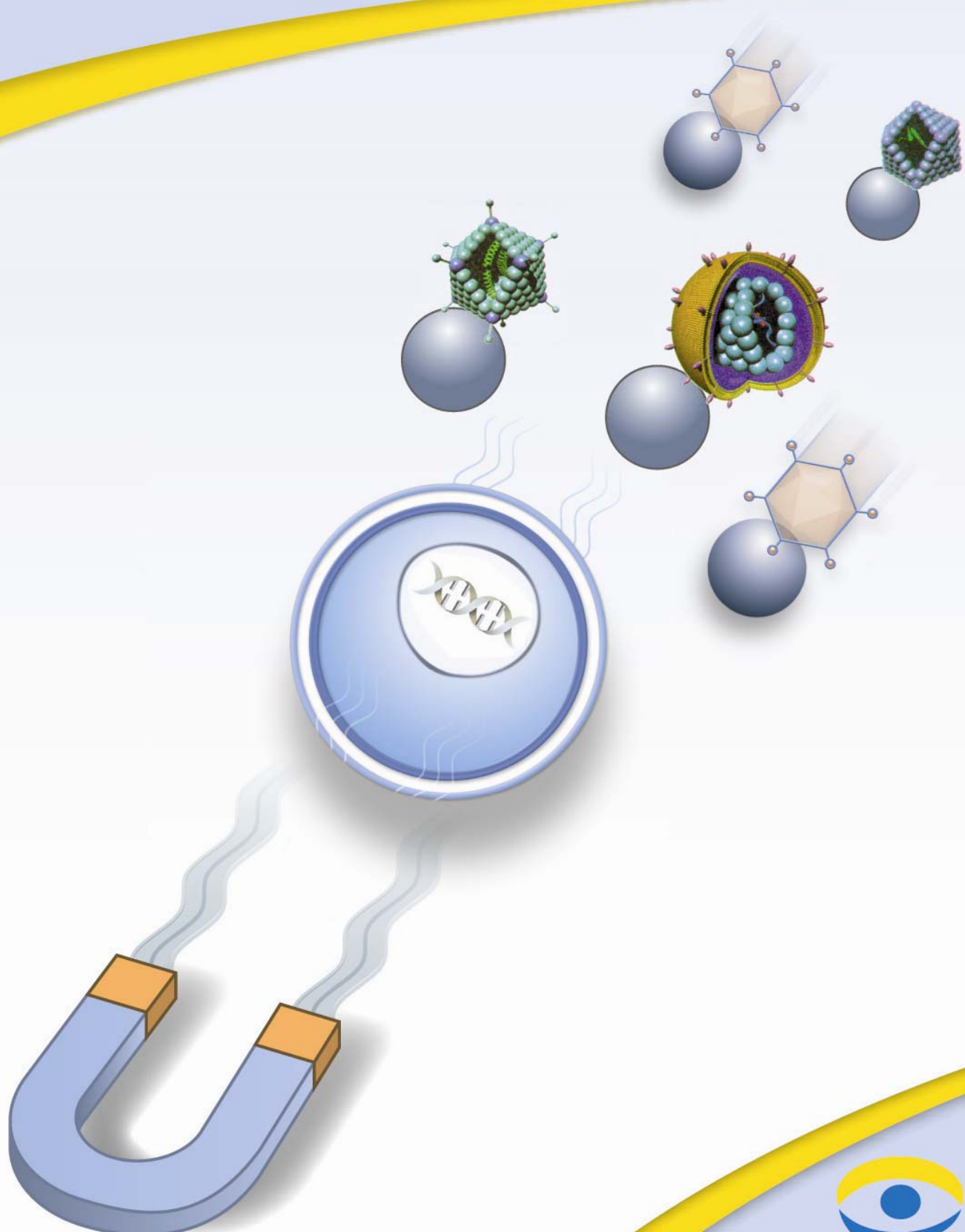


Magnetofection™ - ViroMag & ViroMag R/L

INSTRUCTION MANUAL



ViroMag & ViroMag R/L

Instruction Manual

ViroMag is a superior reagent based on the Magnetofection™* technology suitable for all viral applications.

ViroMag R/L is an improved ViroMag formulation specifically designed for Retrovirus / Lentivirus vectors.

List of ViroMag and ViroMag R/L Kits

Catalog Number	Description	Volume (µL)	Number of transductions / 24 well plates	Number of transductions / 96 well plates
VM40100	ViroMag 100	100	10-100	30-500
VM40200	ViroMag 200	200	20-200	60-1000
VM41000	ViroMag 1000	1000	100-1000	300-5000
RL40100	ViroMag R/L 100	100	10-100	30-500
RL40200	ViroMag R/L 200	200	20-200	60-1000
RL41000	ViroMag R/L 1000	1000	100-1000	300-5000
MF10096	Magnetic Plate with 96-magnets	-		
MF10000	Super Magnetic Plate	-		
KC30500	ViroMag Starting Kit ¹	200	20-200	60-1000
KC30596	ViroMag Starting Kit ²	200	20-200	60-1000

¹ Contains 1 vial of ViroMag VM40200 and a Super Magnetic Plate MF10000

² Contains 1 vial of ViroMag VM40200 and a Magnetic Plate with 96-magnets MF10096

Use the content of the table above to determine the appropriate catalog number for your needs. You can order these products by contacting us. For all other supplementary information, do not hesitate to contact our dedicated technical support (tech@ozbiosciences.com).

OZ BIOSCIENCES

Parc Scientifique et Technologique de Luminy; BP13
13273 Marseille Cedex 9, France
Tel: +33 (0) 491 828 175
Fax: +33 (0) 491 828 170
E-mail: contact@ozbiosciences.com
Web Site: www.ozbiosciences.com

* Patent Pending



1. Technology

1.1. Description

Congratulations on your purchase of the **ViroMag** and/or **ViroMag R/L** reagent!

ViroMag is a specific formulation, issued from our Magnetofection™ technology, designed to be used in association with all viral vectors and for many transduction applications. **ViroMag R/L** is an optimized nanoparticles formulation specifically developed for Retrovirus/Lentivirus. For the first time, scientists will be able to increase transduction efficiency, infect non permissive cells, concentrate virus onto cells or in culture medium, accelerate infection process or synchronize infection without modification of the viruses, just by associating **ViroMag** or **ViroMag R/L** reagents to the viral vectors. **ViroMag** and **ViroMag R/L** are the only reagents available offering a solution to such applications.

Magnetofection™ is a novel, simple and highly efficient viral and non-viral gene delivery method. It exploits magnetic force exerted upon gene vectors associated with magnetic particles to drive the nucleic acids or virus towards, possibly even into, the target cells. In this manner, the complete applied nucleic acid and viral dose gets concentrated on the cells within a few minutes so that 100% of the cells get in contact with a significant vector dose. **ViroMag** and **ViroMag R/L** are exclusive and specific reagents dedicated to viral applications. These reagents demonstrate an exceptionally high efficiency to promote, control and assist viral transductions.

ViroMag is applicable to all viral vectors, **ViroMag R/L** is dedicated to Retrovirus/Lentivirus and they present unique properties allowing to:

1. Increase transduction efficiency in terms of percentage of transduced cells
2. Concentrate all viral dose on the cells very rapidly
3. Accelerate the transduction process.
4. Infect non permissive cells
5. Significantly improve virus infectivity with extremely low vector doses.
6. Synchronize cell adsorption / infection
7. Target / confine transduction to specific area (magnetic targeting)

Based upon a validated and recognized magnetic drug targeting technology this innovative method is:

- Highly Efficient
- Suitable for all viruses
- Economical, Simple & Rapid
- Universal (primary cells, hard-to-transfect cells and cell lines)
- Serum compatible & Non toxic
- Amenable to high throughput automation

1.2. Kit Contents

Kit contents vary according to their size:

- 1 tube containing 0.1 mL of **ViroMag** or **ViroMag R/L** good for 10 to 100 assays in a 24-well plate.
- 1 tube containing 0.2 mL of **ViroMag** or **ViroMag R/L** good for 20 to 200 assays in a 24-well plate.
- 1 tube containing 1 mL of **ViroMag** or **ViroMag R/L** good for 100 to 1000 assays in a 24-well plate.

Stability and Storage

Storage +4°C. Upon receipt and for long-term use, store all tubes in the fridge. Magnetofection kits are stable for at least one year at the recommended storage temperature.

- **DO NOT FREEZE THE MAGNETIC NANOPARTICLES!**
- **DO NOT ADD ANYTHING TO THE STOCK SOLUTION OF MAGNETIC NANOPARTICLES!**

Shipping condition Room Temperature

2. Applications

2.1. Cell Types

ViroMag and **ViroMag R/L** are generally applicable on numerous cell types. This technology has been tested successfully on a variety of immortalized and primary cells. If a particular cell type is not listed, this does not imply that these reagents are not going to work. OZ Biosciences is maintaining an updated list of cells successfully tested available on the website: www.ozbiosciences.com.

<i>Cell Line</i>	<i>Cell Type</i>	<i>Source</i>
293, HEK-293, 293-T, -EBNA	Transformed embryonic kidney	Human
181RDB	Pancreatic cells	Human
A549	Non-small cell lung carcinoma	Human
BT4C	Glioma cells	Rat
B95a	B lymphoblastoid	Simian (Marmoset)
C6	Glioma cells	Rat
CHO-K1	Epithelial-like (ovary)	Hamster
COLO 205	Colon adenocarcinoma	Human
COS-7	Fibroblast (kidney)	Green Monkey
CV-1	Fibroblast-like (kidney)	Monkey
D-17P4	Osteosarcoma	Canine
HeLa	Cervical epithelial carcinoma	Human
HT1080	Fibrosarcoma	Human
HUVEC	Endothelial cells (primary)	Human
K-562	Myelogenous leukemia	Human
L	Fibrosarcoma	Mouse
MDCK	Normal -kidney	Canine
NIH3T3	Fibroblasts	Mouse
PC-12	Pheochromocytoma (adrenal)	Rat
SKOV-3	Ovarian carcinoma	Human
Vero	Fibroblast (kidney)	Green Monkey
Primary aortic endothelial cells (PAEC)		Human, Bovine, Rat
Primary aortic smooth muscle cells		Rabbit
Primary keratinocytes		Human, Mouse
Primary peripheral blood lymphocytes		Human, Mouse
Primary muscle cells		Mouse

2.2. Types of Virus

ViroMag reagent can usually be combined with any viruses. **ViroMag R/L** is particularly suitable for Lentivirus / Retrovirus. If a particular virus is not listed, this does not imply that these reagents are not going to work. OZ Biosciences is maintaining an updated list of virus successfully tested that is available on the website: www.ozbiosciences.com.

<i>Virus Type</i>	<i>Virus name</i>	<i>Application</i>
Adenovirus	Ad5 LacZ, Ad5-PEG	Increase transduction, infect non permissive cells
Adeno-Associated Virus		Increase transduction, infect non permissive cells
Lentivirus / Retrovirus	HIV, MuLV, MLV, FIV	Increase infectivity, synchronize infection
Herpes virus	HSV-I	Concentration
Alpha virus	Sindbis virus	Concentration
Baculovirus	Baavi	Increase transduction, targeting
Rhabdovirus	VSV	Concentration
Polyomavirus	SV40	Concentration
Paramyxovirus	Measles	Increase transduction, infect non permissive cells

2.3. Application examples & Bibliographic References

Until now, a universal method enhancing, assisting, controlling and promoting viral gene delivery systems was lacking. Magnetofection™ is the only existing method answering these different needs. Many studies have demonstrated the potential of using Magnetic Particles such as **ViroMag** and **ViroMag R/L** for viral applications. The conducted studies have shown that magnetic particles including **ViroMag** and **ViroMag R/L**:

✓ **increases transduction efficiency** ¹⁻¹¹

The combination of paramagnetic nanoparticles with adenovirus has shown up to 500-fold enhancement of gene expression compared with standard infection. Significant enhancement (up to 70 fold) of the infection of measles virus has been reported as well as for HIV and VSV (about 100 fold increase).

✓ **concentrates viral dose, promotes and accelerates the infection process** ¹⁻¹²

Retroviral titers could be increased by 1000 to 4000 fold. Concentration of measles virus, aav, non-enveloped virus (SV40) and enveloped virus such as Sindbis virus, HSV-I and VSV has been reported. Transduction efficiency of PEGylated adenovirus can be restored by the use of magnetic nanoparticles.

✓ **improves viral infectious capacity** ^{1-3, 7, 9}

Significant enhancement of retrovirus infectivity can be achieved with the use of magnetic nanoparticles.

✓ **extends the host tropisms of viral vectors to non-permissive cells** ^{1, 2, 5, 10}

The association of viral vectors with magnetic nanoparticles is sufficient to force infection of non-permissive cells as shown with adenovirus in NIH 3T3, K562 cells, human peripheral blood lymphocytes, COLO25 and C6 and with the measles virus in SLAM-negative cell lines.

✓ **allows the synchronization of the transduction** ⁷

Synchronized adsorption of HIV-1 on primary cells can be accomplished with the use of magnetic nanoparticles.

✓ **can provide a magnetic targeting.** ^{1, 5, 6, 8-11}

High transduction can be achieved under magnetic influence and a specific targeting to define area can be done. Indeed, magnetic targeting confine to specific area linked to the magnet size and shape has been demonstrated for adenovirus, AAV, baculovirus and retrovirus

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3. ViroMag / Magnetofection™ Apparatus

As for all Magnetofection™ reagents, **ViroMag** and **ViroMag R/L** require an appropriate magnetic field. Two magnetic plates (96-magnets plate and supermagnetic plate) especially designed for Magnetofection to exert this specific magnetic field are available. Their special geometry produce a strong magnetic field that is suitable for all cell culture dishes (T-75 flasks, 60 & 100 mm dishes, 6-, 12- 24-, 48- and 96-well plates).



4.1. General Considerations

The instructions given below represent sample protocols that were applied successfully with a variety of cells and viruses. Our R&D team has tested and optimized the **ViroMag** and **ViroMag R/L** reagents in order to provide you with the straightforward and efficient procedure. Therefore, we recommend you to start by following our general protocol as guidelines to obtain good data quickly and if necessary, we advise you to optimize the experimental conditions parameters in order to achieve the best effects. Optimal conditions do vary from cell to cell and are highly dependent upon the type of virus used, its titer, the composition of the viral solution, and cell culture conditions. Consequently, the amount, concentration and ratio of the individual components (virus and ViroMag), the time course and the number of cells may have to be adjusted to get the best results. Several optimization protocols are available in the Appendix.

4.2. Cell Culture

It is recommended to seed or plate the cells the day prior transduction, however cells can also be prepared few hours before the transduction. Suspension cells should be prepared in the adequate vessel just before the infection (see below for specific protocol). The suitable cell density will depend on the growth rate and the condition of the cells. Best results are achieved if cells are at least 60-80 % confluent at the time of Magnetofection (see the suggested cell number in the table below). 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.

Table 1: Recommended cell number.

Culture vessel	Number of adherent cells	Number of suspension cells	Final Transduction Volume*
96-well	0.05 – 0.15 x 10 ⁵	0.5 – 1 x 10 ⁵	150 µL
24-well	0.5 – 1 x 10 ⁵	2 – 5 x 10 ⁵	500 µL
12-well	1 – 2 x 10 ⁵	2.5 – 10 x 10 ⁵	1 mL
6-well	2 – 5 x 10 ⁵	1 – 2 x 10 ⁶	2 mL
60 mm dish	5 – 10 x 10 ⁵	2.5 – 5 x 10 ⁶	4 mL
90 – 100 mm dish	15 – 30 x 10 ⁵	5 – 10 x 10 ⁶	8 mL
T-25 flask	5 – 10 x 10 ⁵	2.5 – 5 x 10 ⁶	5 mL
T-75 flask	20 – 50 x 10 ⁵	5 – 15 x 10 ⁶	10 mL

*Transduction volume corresponds to the volume of culture medium covering the cells plus the volume of the ViroMag/virus mixture.

According to the standard protocol, the virus / **ViroMag** or **ViroMag R/L** mixtures are prepared in medium with or without serum and supplement or in physiological saline. These mixtures are then added to the cells that are covered with complete medium. Therefore, the addition of this cocktail will result in the dilution of supplements such as serum, antibiotics or other additives of your standard culture medium if medium without serum and supplement or physiological saline is used. Although a medium change after Magnetofection is not required for most cell types, it may be necessary for cells that are sensitive to serum/supplement concentration. If cell culture viral supernatant is used instead, you can also replace the cell culture medium by that one.

4.3. ViroMag Procedure

Viral Magnetofection is carried out in the same manner as standard transductions with the following exceptions:

- Virus preparations are mixed with **ViroMag** or **ViroMag R/L** prior to transduction
- Cell culture plate is positioned upon the magnetic plate during transduction
- Polybrene or other additives are NOT used for transductions.

The protocol is straightforward. For instance, 30 µL of **ViroMag** magnetic particles have been found sufficient to bind 10 billion of viral particles. Thus, the particle amounts listed in **Table 2** will be mainly sufficient to bind virus doses which are usually applied in transduction experiments. Depending on the viral vector type, the quantity of virus and the cell type used, this protocol would have to be adjusted (see appendix for optimization protocol). **ViroMag R/L** is an improved formulation of **ViroMag**, specifically designed for Retrovirus and Lentivirus, and should be used the same way as **ViroMag**.

- 1) Plate the cells the day before infection or just before infection in your appropriate tissue culture dish as suggested in Table 1.
- 2) Add a suitable amount (see table below) of **ViroMag** or **ViroMag R/L** in a tube large enough to contain the volume of virus preparation added in step 3. If required, **ViroMag** & **ViroMag R/L** can only be diluted with deionized water. Do not dilute the reagents in serum and supplement-free medium. The amount of **ViroMag** or **ViroMag R/L** depends on the type and dose of virus used. As a starting point, the "suggested ViroMag quantity" indicated in the table 2 can be used. However, we highly recommend adjusting the amount of **ViroMag**. For example, use 1.5 μL , 3 μL , 6 μL , and 12 μL of **ViroMag** or **ViroMag R/L** with a fixed quantity of virus preparation / supernatant in 24-well. Refer to Table 2 for the other ranges of dose.
- 3) Add your virus preparation to the tube(s) containing **ViroMag** or **ViroMag R/L** and mix immediately by pipetting up and down. Virus preparation is preferably in serum free medium or salt-containing buffers.

Note 1: If required, dilute the aliquot of your virus preparation to be used for transduction with serum-free cell culture medium or other salt-containing buffer (e.g. retroviral supernatant or purified adenovirus diluted in HBS, PBS or cell culture medium). Alternatively, you can directly use an aliquot of culture supernatant from a producer cell line

Note 2: The ratios virus / **ViroMag** should be adjusted according to the viral titers and cell types used.

Table2: Recommended amount of **ViroMag** & **ViroMag R/L**, volume of vector preparation and final transduction volume:

Culture Vessel	ViroMag Quantity (μL)	Suggested ViroMag Quantity (μL)	Volume of ViroMag/virus solution	Final Transduction Volume*
96 well	0.2 – 3	1.5	50 μL	150 μL
24 well	1 - 12	6	100 μL	500 μL
12 well	2 - 24	12	100 μL	1 mL
6 well	5 - 60	30	200 μL	2 mL
60 mm dish	10 - 120	60	400 μL	4 mL
90 - 100 mm dish	30 - 300	150	800 μL	8 mL
T-25 flask	10 - 120	60	500 μL	5 mL
T-75 flask	30 - 300	150	1000 μL	10 mL

*Transduction volume corresponds to the volume of culture medium covering the cells plus the volume of the ViroMag/virus mixture

Table3: Successful examples of **ViroMag** and **ViroMag R/L** experimental procedure

Cell types	Virus type	Titer (<i>MOI, CFU, TCID₅₀</i>)	ViroMag Quantity (μL)	Culture Vessel
K562	adenovirus	200 MOI	12 μL	24 well
NIH-3T3	adenovirus	200 MOI	6 - 12 μL	6 well
PBL	adenovirus	500 MOI	3 - 6 μL	96 well
NIH-3T3	Retrovirus (MuLV)	1-5 x 10 ³ CFU/mL	6 - 12 μL	6 well
HeLa, NIH3T3, K562, HEK293...	VSV-G pseudo -HIV	0.5, 1 and 2 MOI	5 - 7 μL	24 well
U87 CD4+	HIV-1	Not know	10% (v/v)	96-well
Vero, B95a, HeLa, L & CHO	Measles virus	5 x 10 ² TCID ₅₀	2 μL	96 well
PAEC, NIH-3T3	HIV-1, MuLV, lenti-VSV	3x10 ³ - 2x10 ⁴ IU	5 - 10 μL	12 well

- 4) Incubate 5 to 15 minutes either at room temperature or on ice.
- 5) Add the **ViroMag** or **ViroMag R/L** / virus mixture to the cells to be transduced.
- 6) Place the cell culture plate upon the magnetic plate for 15 minutes. Longer incubation time (30 or 60 minutes) or shorter (1 to 5 minutes for synchronization) can also be used.
- 7) Remove the magnetic plate and cultivate the cells under standard conditions until evaluation of the transduction experiment. Optionally perform a medium change.

4.4. Suspension Cells Protocol

1. The composition and preparation of **ViroMag** / virus or **ViroMag R/L** / virus mixtures are performed exactly as described above from steps 1 to 4 (section 4.3 pages 5 and 6).
2. While the **ViroMag** (or **ViroMag R/L**) / virus mixtures incubate (step 4 above), prepare the cells to be transduced (as suggested in **Table 1**). For example, dilute the cells to 5×10^5 - 1×10^6 / 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.
 - a. Seed the cells on polylysine-coated plates and use the protocol for adherent cells, **OR**
 - b. Briefly, centrifuge the cells (2 minutes) to pellet them and use the protocol for adherent cells, **OR**
 - c. Mix cell suspension with 20-30 μ L of **CombiMag** reagent (Magnetofection) per 1 ml of cell suspension and incubate for 10 - 15 minutes. Then, distribute the cells to your tissue culture dish placed upon the magnetic plate and incubate for 15 more minutes
3. Add the resulting mixture of **ViroMag** (or **ViroMag R/L**) / virus to the cells while keeping the cell culture plate on the magnetic plate.
4. Continue to incubate for 15 minutes.
5. Remove culture plate from magnetic plate.
6. Continue to cultivate cells as desired until evaluation of the transduction experiment.

5. Appendix

5.1. Critical Parameter for best performance

- 1) Cell culture conditions: Best results are achieved when cells are 60–80 % confluent at the time of the transduction. If necessary, you can wash the culture medium containing the transduction mixture after 8-24 hours and replace it by fresh medium. However, cells should be plated as required for standard viral gene delivery. The density can be high for adenovirus but must be low for retrovirus.
- 2) ViroMag or ViroMag R/L quantity. We often observed good effects at very low doses of ViroMag (2-3 μ L / well for a 24-well plate). However the efficiency may depend on the cell line and the virus type used. Consequently, we suggest you to start by testing a range of ViroMag volumes in order to obtain the best experimental conditions.
- 3) Time course. The infection time course depends on the amount/concentration of virus used. Indeed, longer incubation under the magnetic field is required with very low viral titers whereas with high viral dose short incubation times are sufficient.

5.2. Protocol Optimization

In order to get the best out of **ViroMag** and **ViroMag R/L**, several parameters can be optimized:

- ViroMag dose & Ratio of ViroMag to Virus
- Cell type, cell density and incubation times

OZ Biosciences team has investigated numerous factors during the course of the R&D program. Based on our experience, we recommend that you optimize one parameter at a time and start from the experimental procedures described above (section 4).

- 1) Start by optimizing the **ViroMag** or **ViroMag R/L** dose with a **fixed amount of virus**. This will vary the concentration of ViroMag and the ratio ViroMag / Virus. To this end, vary the amount of ViroMag in the range suggested in the Table 2. For instance, from 0.2 to 3 μ L of ViroMag or ViroMag R/L in a 96-well plate.
- 2) Next, you can inverse the procedure by optimizing the dose of virus with a **fixed amount of reagent**.
- 3) After having identified the correct quantity of **ViroMag** or **ViroMag R/L** and virus, you could pursue the process by optimizing the **cell number** (density) and **time course of incubation**, between **ViroMag** and viruses (section 4.3.4) and under the magnetic plate (section 4.3.6).

5.3. Quality Controls

To assure the performance of each lot of **ViroMag** & **ViroMag R/L** produced, we qualify each lot using rigorous standards. *In vitro* assays are conducted to qualify the quality and activity of each kit component.

Components	Standard Quality Controls
ViroMag & ViroMag R/L	<ol style="list-style-type: none">1. Quality and size homogeneity of the magnetic nanoparticles.2. Stability of the magnetic nanoparticles formulations.3. ViroMag transduction efficacies with a recombinant adenovirus on NIH-3T3 cells. Every lot shall have an acceptance specification of > 80% of the activity of the reference lot.4. ViroMag R/L transduction efficacies with a recombinant pseudo HIV (GFP) on HeLa cells. Every lot shall have an acceptance specification of > 80% of the activity of the reference lot.5. Sterility. Thioglycolate assay: absence of fungal and bacterial contamination shall be obtained for 7 days.
Magnetic Plate	<ol style="list-style-type: none">1. Tests of solidity and Test of the magnetic field force

5.4. "Troubleshooting"

Our dedicated and specialized technical support team will be pleased to answer any of your requests and to help you with your transfection experiments at tech@ozbiosciences.com. In addition, do not hesitate to visit our website www.ozbiosciences.com and the FAQ section.

6. Related Products

OZ Biosciences offers three other types of ready-to-use **Magnetofection™** reagents.

1. **PolyMag** designed for all transfection applications and all nucleic acids.
2. **CombiMag** aimed to be combined with any transfection reagent for enhancing transfection efficiency.
3. **SilenceMag** created specifically for all siRNA applications.

Description	Reference
Magnetofection™ Starting Kit ¹	KC30296
Magnetofection™ Super Starting Kit ²	KC30496
PolyMag – 200 µL	PN30200
PolyMag – 1000 µL	PN31000
CombiMag – 200 µL	CM20200
CombiMag – 1000 µL	CM21000
SilenceMag – 200 µL	SM10200
SilenceMag – 1000 µL	SM11000
DreamFect™ – 1 mL	DF41000
DreamFect™ – 5 x 1 mL	DF45000
EcoTransfect – 1 mL	ET11000
VeroFect – 1 mL	VF61000
FlyFectin™ – 1 mL	FF51000
GeneBlaster™ Ruby	GB20011
GeneBlaster™ Sapphire	GB20012
GeneBlaster™ Topaz	GB20013
β-Galactosidase (ONPG) assay kits	GO10001
β-Galactosidase (CPRG) assay kits	GC10002
X-Gal staining kit	GX10003

¹ Contain: PN30100 + CM20100 + MF10096

² Contain: PN30100 + CM20100 + SM10200 + MF10096

Please, feel free to contact us for all complementary information and remember to visit our website to stay informed on the latest breakthrough technologies and updated on our complete product list.

Limited License

The purchase of the ViroMag, ViroMag R/L and other Magnetofection™ Reagents grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in section 1, Kit Contents). This reagent is intended **for in-house research only** by the buyer. Such use is limited to the transfection and transduction of nucleic acids and virus as described in the product manual. In addition, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences.

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The ViroMag, ViroMag R/L and other 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 included/used in any drug intended for human use. All care and attention should be exercised in the use of the kit components by following proper research laboratory practices.

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Director of Business Development
OZ Biosciences
Parc Scientifique et Technologique de Luminy
BP13
13273 Marseille Cedex 9, France
Tel: +33 (0)4.91.82.81.74
Fax: +33 (0)4.91.82.81.70
E-mail: contact@ozbiosciences.com