ViroMag R/L Results

OZ Biosciences is delighted to announce the launching of a new product based on the Magnetofection ™ technology, specifically designed for ***Lentiviral and Retroviral*** application: **ViroMag R/L**. Magnetofection™ uses magnetic force to drive the virus associated with magnetic particles towards and into the target cells. In this way, the complete applied dose of virus gets concentrated onto the cells surface very rapidly so that 100% of the cells get in contact simultaneously with all viral doses.

ViroMag R/L is applicable to all retroviral and lentiviral vectors and present unique properties allowing to:

1. Increase transduction efficiency in terms of percentage of transduced cells
2. Concentrate the entire viral dose on the cells very rapidly
3. Accelerate the transduction process.
4. Significantly improve virus infectivity with extremely low vector doses.
5. Synchronize cell adsorption / infection without modification of the viruses
6. Target/confine transduction to specific area (magnetic targeting)

ViroMag and **ViroMag R/L** are the only reagents available offering a solution to such applications. **ViroMag R/L** and virus to be transduced are mixed in a one-step procedure; no molecular biology process or biochemical modifications are required. This reagent demonstrates an exceptionally high efficiency to promote, control and assist ***Lentiviral and Retroviral*** transductions.

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

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

OZ Biosciences offers 6 types of ready-to-use reagents:

- ✓ **ViroMag R/L** specifically designed for retroviral and lentiviral vectors
- ✓ **ViroMag** engineered to be combined with all viruses
- ✓ **PolyMag** suitable for all nucleic acids and all transfection application
- ✓ **CombiMag** designed to be associated with all transfection reagents
- ✓ **SilenceMag** created specifically for all siRNA applications.
- ✓ **FluoMag** (fluorescent nanoparticles) developed to track and analyze delivery (biodistribution, cytometry, microscopy, mechanisms...)

Virus Types

ViroMag R/L reagent can be combined with any retroviruses and lentiviruses such as: HIV, MuLv, MLV, FIV, and SIV. If a particular virus is not listed, this does not imply that **ViroMag R/L** is not going to work.

Cell Types

ViroMag R/L is applicable and has been tested successfully on a variety of cells such as: HEK293, CHO, HeLa, COS, K562, NIH3T3, VERO, BT4C, U87...).

ViroMag R/L is generally applicable on numerous cell types, but if a particular cell type is not listed, this does not imply that **ViroMag R/L** is not going to work. OZ Biosciences is going to frequently update this list.

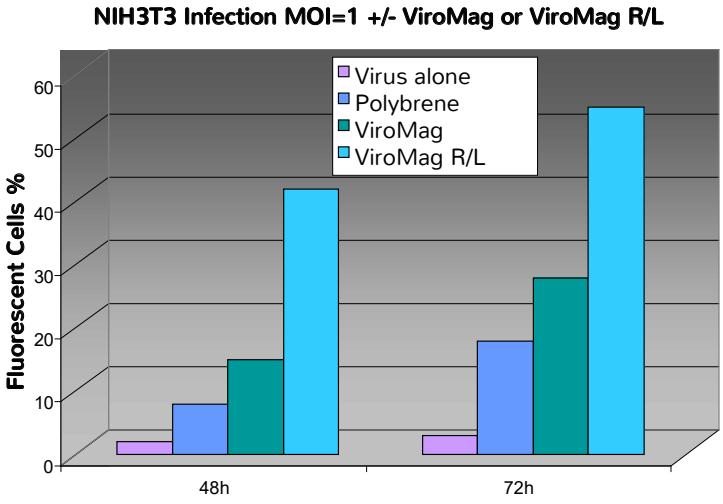
Applications and Results

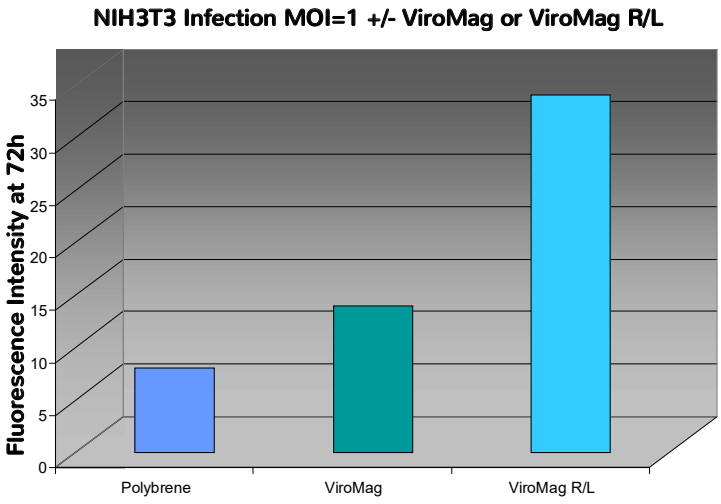
ViroMag R/L increases transduction efficiency

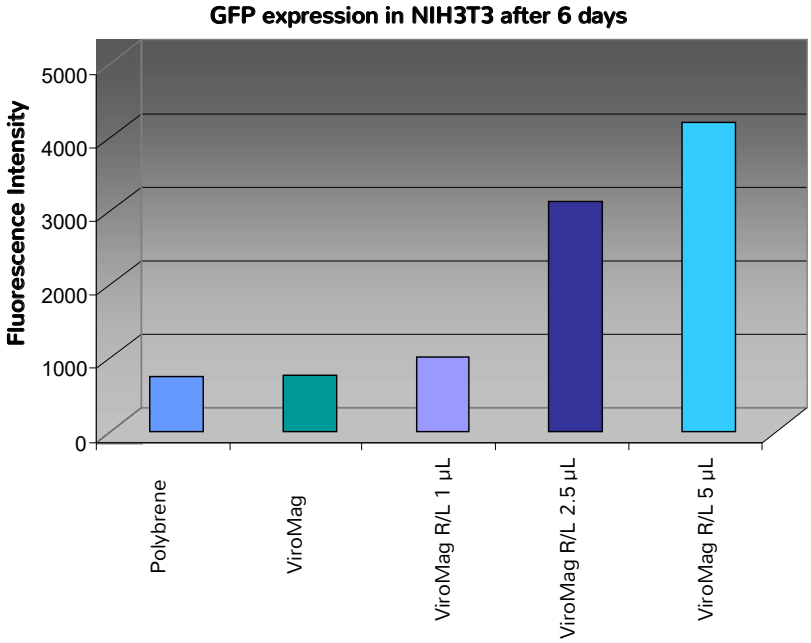
1) ***HIV based*** *(*VSV-G pseudo-typed viral particles) **retrovirus**

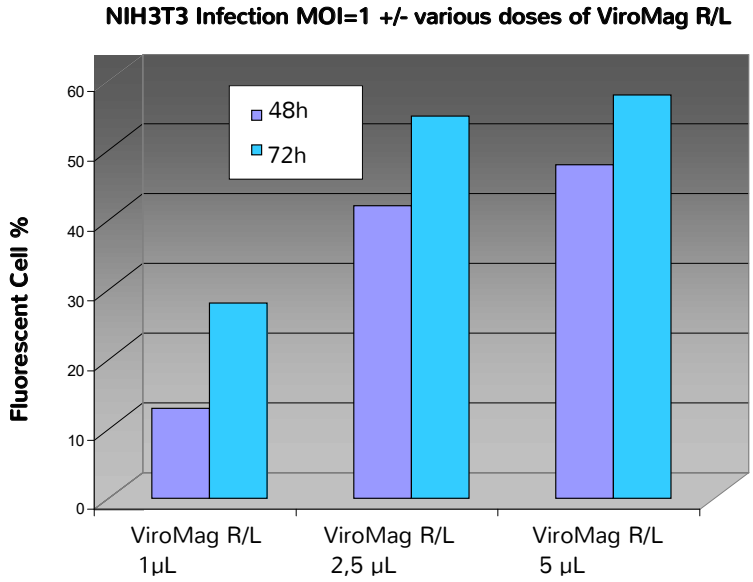
Transduction efficiency of pseudo-typed HIV viruses carrying a GFP reporter gene were assayed in NIH-3T3 and HeLa cells. Cells were plated the day before infection in a 24-well plate, and were then infected with 0.5 or 1 MOI either in presence of 5 µg/mL polybrene or in presence of 5µL of ViroMag or ViroMag R/L. After 15 min of Magnetofection (incubation of virus/ViroMag complexes with cells on a magnetic plate) as indicated in ViroMag protocol, cells were placed in a 5% CO_2 incubator at 37°C. Culture medium was replaced 24h post-infection by fresh culture medium. Cells were observed at 24, 48, 72h and 6 days after the infection. GFP expression was analysed by FACS at various time in term of GFP positive cells (%) and GFP fluorescence intensity

NIH-3T3 cells

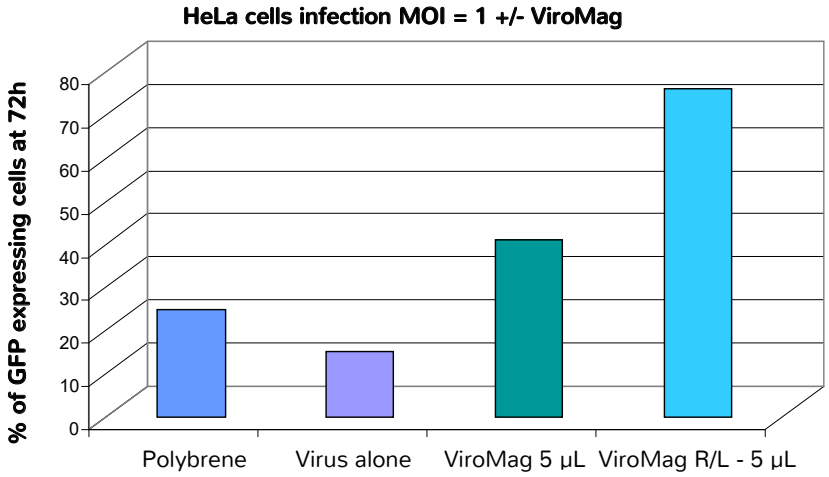


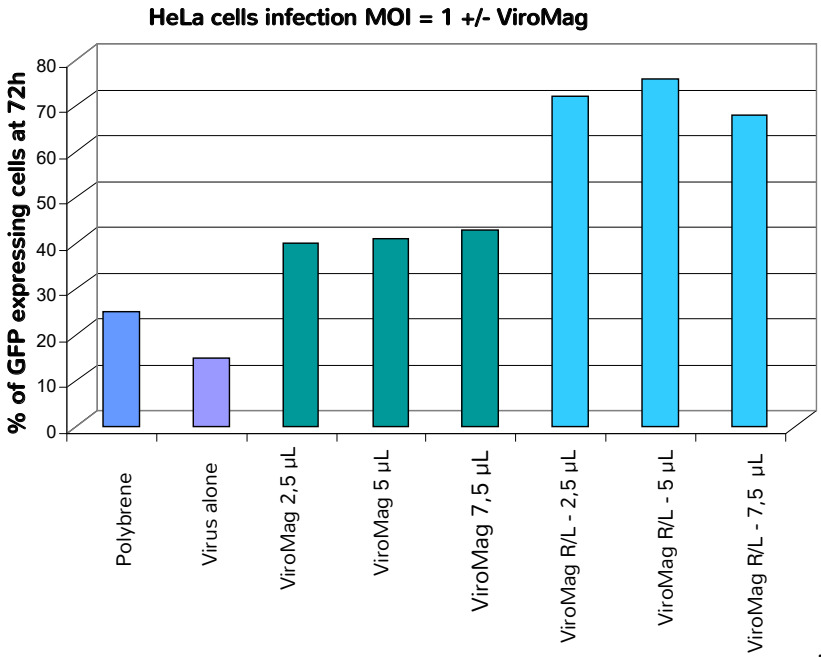


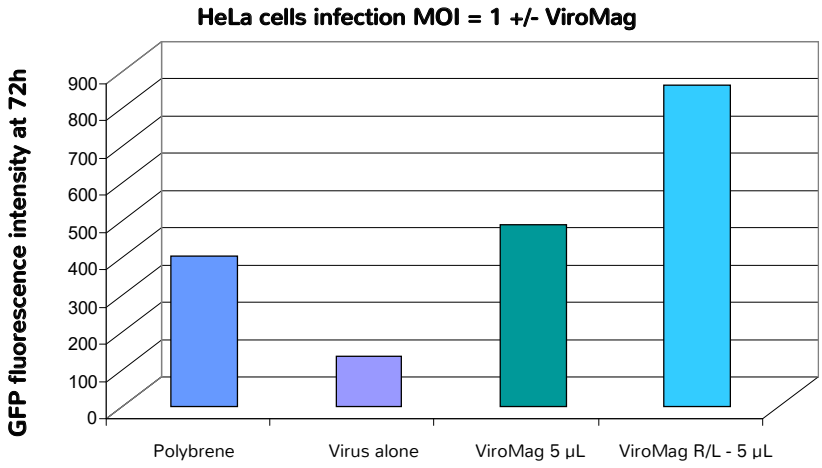



NIH3T3 Infection MOI=1 +/- various doses of ViroMag R/L
Fluorescent Cell %
48h
72h
0
10
20
30
40
50
60
ViroMag R/L 1µL
ViroMag R/L 2,5 µL
ViroMag R/L 5 µL

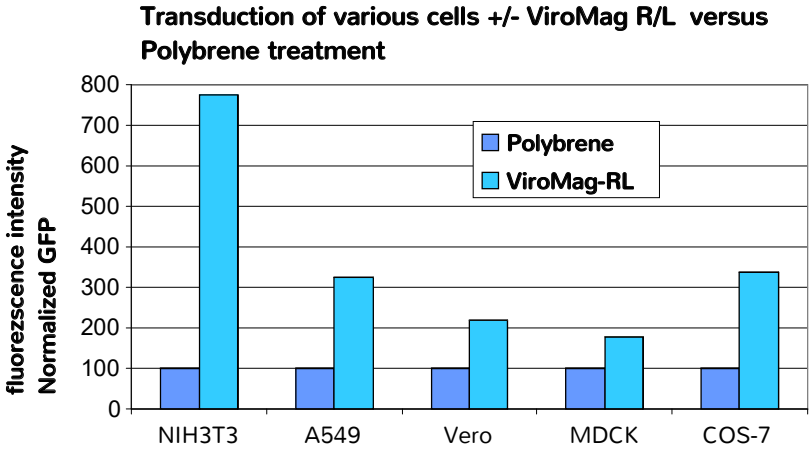
HeLa ce

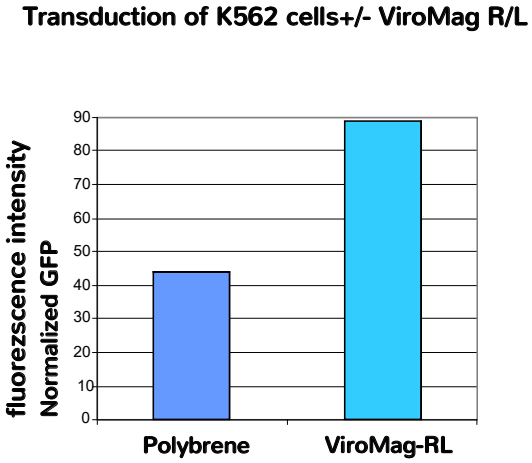

HeLa cells infection MOI = 1 +/- ViroMag
% of GFP expressing cells at 72h
0
10
20
30
40
50
60
70
80
Polybrene
Virus alone
ViroMag 5 µL
ViroMag R/L - 5 µL


HeLa cells infection MOI = 1 +/- ViroMag
% of GFP expressing cells at 72h
0
10
20
30
40
50
60
70
80
Polybrene
Virus alone
ViroMag 2,5 µL
ViroMag 5 µL
ViroMag 7,5 µL
ViroMag R/L - 2,5 µL
ViroMag R/L - 5 µL
ViroMag R/L - 7,5 µL

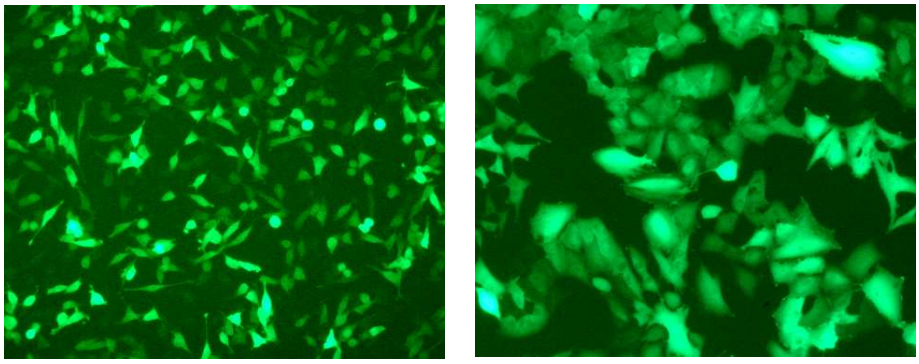


Various cell lines





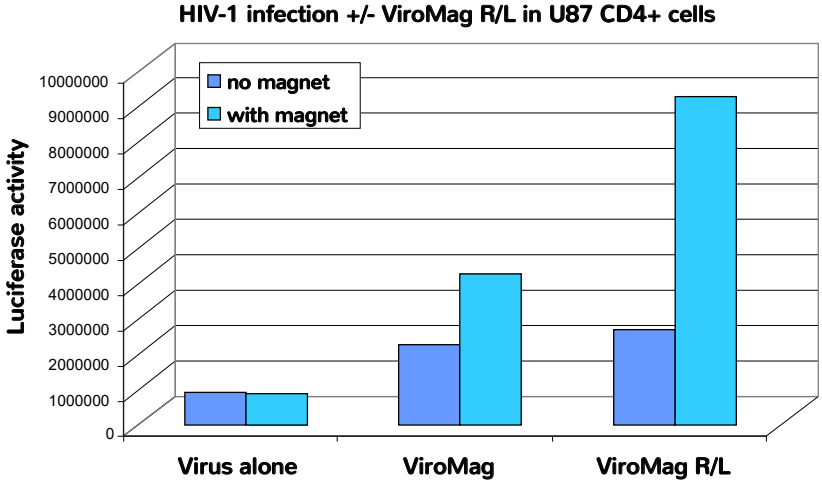
HeLa **A549**



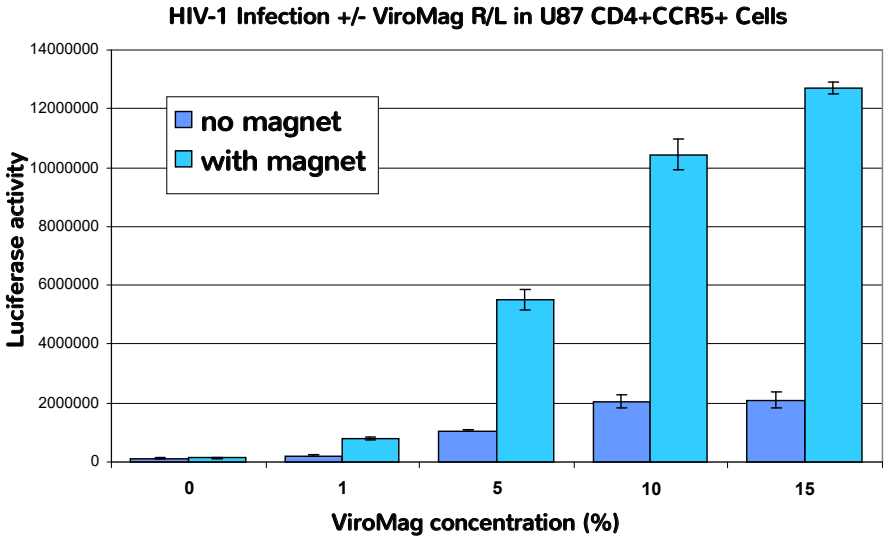
Hela and A549 cells transduced with a pseudo-typed HIV viruses carrying a GFP reporter gene plus **ViroMag R/L** 48 hours post-infection

2) **HIV pseudo-virus**

– Pseudo-typed HIV-I viruses carrying a luciferase reporter gene were produced in 293 cells. Supernatants containing the recombinant HIV-1-Luc viruses (rHIV-Luc) were associated with ***ViroMag*** or ***ViroMag* R/*L*** at a volume of 5% (5µL ViroMag in 100µL of viral supernatants). This represents a ratio of 50µL of ***ViroMag R/L*** per mL of rHIV-Luc supernatant. Mixtures were added to U87-CD4-CCR5 cells growing in 96-well plates (0.5×10^5 cells / well), incubate or not 30 minutes on magnetic plates, the medium was then replaced and luciferase activity was measured at 72 hours post-transduction. U87-CD4+ cells were infected with a rHIV-1-Luc +/- ViroMag R/L and +/- the magnetic field. ***ViroMag R/L*** clearly increased the HIV-1 infection efficiency as shown in figure below.



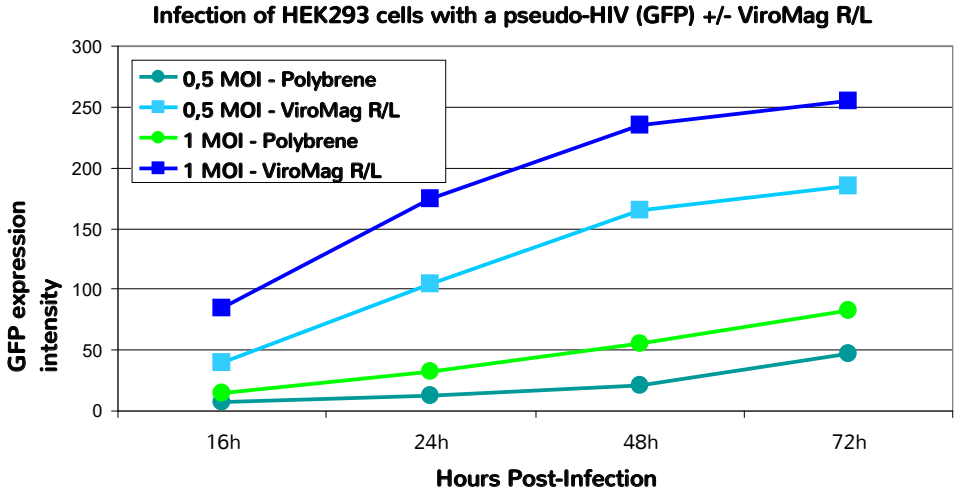
– rHIV-Luc were produced in 293 cells. Supernatants containing the rHIV-Luc viruses were associated with various amounts of ***ViroMag* R/*L*** *(concentration shown as % of viral supernatant volume used for complexation with ViroMag R/L)*. For instance, 10% means 10µL of **ViroMag R/L** in 100µL of viral supernatants. Mixtures were added to U87-CD4+ cells, incubate 30 minutes on magnetic plates, the medium was then replaced and luciferase activity was measured at 72 hours.

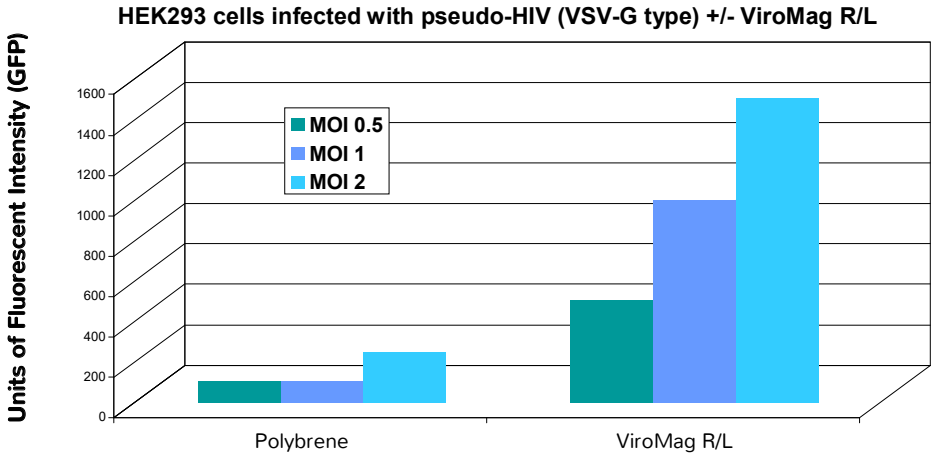


ViroMag concentrates viral dose, promotes and accelerates the infection process

1) ***HIV based*** (VSV-G pseudo-typed viral particles) **retrovirus**

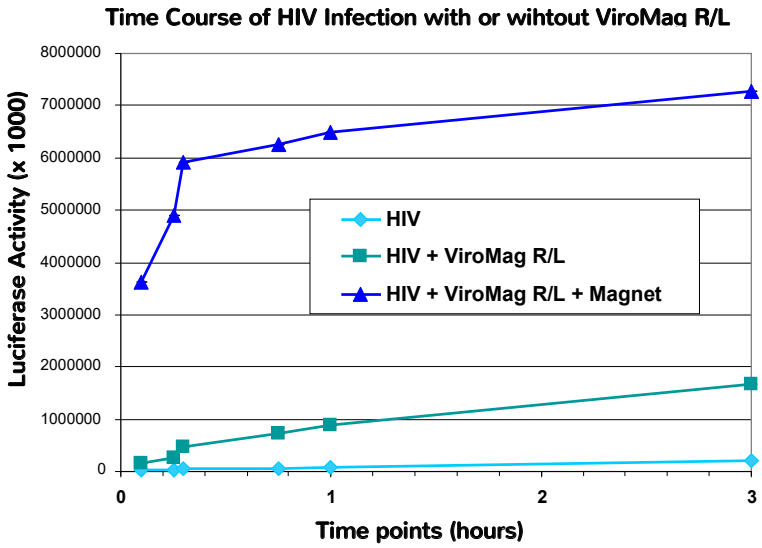
Transduction efficiency of pseudo-typed HIV viruses carrying a GFP reporter gene were assayed in HEK293 cells. Cells were plated the day before infection in a 24-well plate, and were then infected with 0.5 or 1 MOI either in presence of 5 µg/mL polybrene or in presence of 5µL of ViroMag R/L. After 30 min of Magnetofection (incubation of virus/ViroMag complexes with cells on a magnetic plate) as indicated in ViroMag protocol, cells were placed in a 5% CO_2 incubator at 37°C. Culture medium was replaced 24h post-infection by fresh culture medium. Cells were observed at 16, 24, 48 and 72h after the infection. GFP expression level was monitored with a fluorometer.





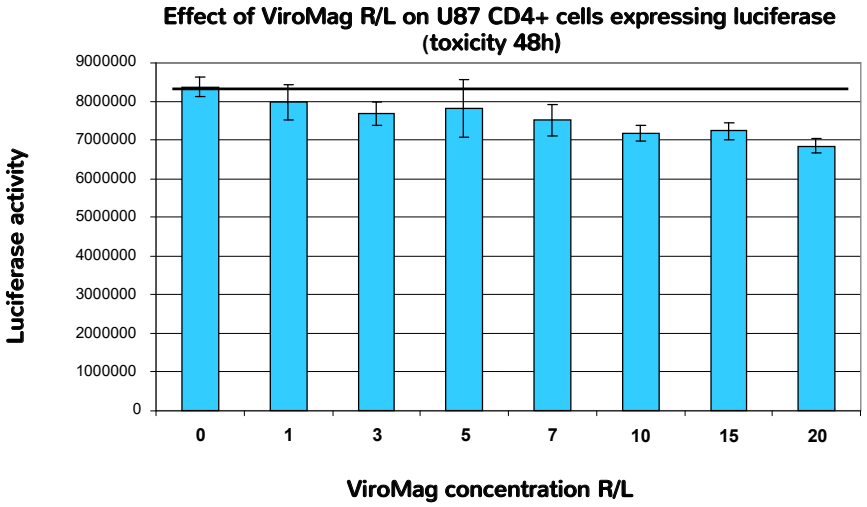
2) **HIV pseudo-virus**.

Concentration and acceleration of the infection process has also been demonstrated with a pseudo-typed HIV-I virus carrying a luciferase reporter gene (rHIV-Luc). rHIV-Luc were produced in 293 cells. Supernatants containing the recombinant HIV-1-Luc viruses (rHIV-Luc) were associated or not with ***ViroMag*** at a ratio of 100 µL of ***ViroMag*** per mL of rHIV-Luc supernatant (10% volume ratio). U87-CD4+ cells were infected with a rHIV-1-Luc +/- ViroMag R/L and +/- the magnetic field. At various incubation time, cell culture were washed and replaced with fresh culture medium. Then, Luciferase expression was monitored at 48h.



ViroMag R/L effect on cells expressing luciferase

U87-CD4+ cells were infected with pseudo-typed HIV-I viruses carrying a luciferase reporter gene and incubate for 24 hours. Cells expressing luciferase were then incubated with various amount of ***ViroMag R/L*** for 48 hours. ViroMag concentration is reported as a % of ViroMag volume used for the mixing with viral supernatant. For instance, 10% means 10µL of ViroMag R/L in 100µL of culture medium. Luciferase activity was measured at 48 hours after addition of nanoparticles.



Examples of Viral (Retro- & Lentivirus) applications with Magnetic nanoparticles

1. **Increase Transduction Efficiency**
   a. The infectivity of lentiviruses (HIV-1 and a pseudo lenti-VSVG) has been shown to be increased by about 100-fold when the virus were adsorbed on magnetic nanoparticles [1].
   b. A magnetic retroviral vectors formed by the combination of paramagnetic nanoparticles and a Retrovirus (Moloney Leukemia Virus) demonstrated major higher gene transduction efficiency [2].

2. **Concentrate viral dose and accelerate the infection process**
   a. Concentration of viruses from cell culture supernatants has been reported wherein retroviral titers could be increased by 1000 to 4000 fold [3].
   b. The rate of retroviral infection is primarily limited by diffusion-dependent cell association. Association of a Lentivirus (HIV-I) with magnetic nanoparticles has led to a considerable concentration of the viral dose on cell surface [1]. Cellular uptake of HIV-1 was increased by 70-fold.

3. **Improve viral infectious capacity**
   a. Enhancement of infectivity with ***ViroMag***. Low retroviral titer preparation was associated to ***ViroMag*** and used to transduce NIH-3T3 cells [4, 5, 6]. No transductions were observed with virus alone, Whereas ViroMag led in a 20-fold enhancement over a standard (virus + polybrene) transduction approach.
   b. Improvement of retrovirus infectivity was also demonstrated by *Hughes et al*. 20 times infectivity enhancement was achieved with a high retroviral titer when combined to paramagnetic particles [3].
   c. The infectivity of a lentivirus was shown to be clearly increased when associated with paramagnetic nanoparticles [1]. This enhancement of infectivity was seen even at low MOI.

4. **Synchronize cell adsorption / infection**
   a. Synchronized infection by HIV-1 of primary endothelial cells was reported with the use of magnetic nanoparticles [1]. When virus was complexed onto paramagnetic nanoparticles optimum cellular uptake was reached after 1 minute. This magnetically controlled viral adsorption is advantageous to synchronize infection and to accurately monitor the kinetics of viral replication cycle.

5. **Magnetic Targeting**
   a. In vitro magnetic targeting has also been demonstrated with Retrovirus [3]. In this manuscript the elegant design of the magnet shape strongly show a confine and specific targeting to the area dictated solely by the presence of the magnet. In another report, site specific delivery was obtained with a magnetic retroviral vector (MLV) [2].

Bibliographic References

1. Haim, H., et al. Synchronized infection of cell cultures by magnetically controlled virus. 2005. *J. Virol.* **79**(1): 622-5.
2. Tail et al. Generation of magnetic retroviral vectors with magnetic nanoparticles. 2003. Rev. Adv. Mater. Sci. 5:319-323
3. Hughes, C., et al. Streptavidin paramagnetic particles provide a choice of three affinity- based capture and magnetic concentration strategies for retroviral vectors. 2001. *Mol. Ther.* **3**(4): 623-30
4. Scherer F, et al. Magnetofection: enhancing and targeting gene delivery by magnetic force in vitro and in vivo. *Gene Ther.* 2002; 9(2):102-9.
5. Plank C, et al. Enhancing and targeting nucleic acid delivery by magnetic force. *Expert Opin Biol Ther.* 2003; 3(5):745-58.
6. Schillinger, U., et al. Advances in Magnetofection – magnetically guided nucleic acid delivery. 2005. *J. Magn. Magn. Mat.* **293**: 501-508.