ViroMag Results

OZ Biosciences is delighted to announce the launching of a new product based on the Magnetofection ™ technology, specifically designed for viral application: **ViroMag**. Magnetofection™ is a new revolutionary nucleic acids delivery system. It exploits magnetic force to drive the nucleic acids vectors and 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** is a versatile and unique solution for many viral applications. ViroMag allows scientist to:

- ✓ Increase transduction efficiency and accelerate transduction process
- ✓ Infect non permissive cells
- ✓ Concentrate virus onto cells or in culture medium
- ✓ Synchronize cell adsorption (infection) without modification of the viruses

ViroMag is the only reagent available offering a solution to such applications. **ViroMag** 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 viral transductions.

ViroMag is applicable to all viral 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 and accelerate the transduction process.
3. Infect non permissive cells
4. Significantly improve virus infectivity with extremely low vector doses.
5. Synchronize cell adsorption / infection
6. 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

OZ Biosciences offers 4 types of ready-to-use reagents:

- ✓ **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.

Virus Types

ViroMag reagent can generally be combined with any viruses. If a particular virus is not listed, this does not imply that **ViroMag** is not going to work.

Virus Type	*Virus name*
Adenovirus / Adeno-Associated Virus	Ad5 LacZ, Ad5-PEG
Lentivirus / Retrovirus	HIV, MuLV, MLV
Herpes virus	HSV-I
Alpha virus	Sindbis virus
Baculovirus	
Rhabdovirus	VSV
Polyomavirus	SV40
Paramyxovirus	Measles

Cell Types

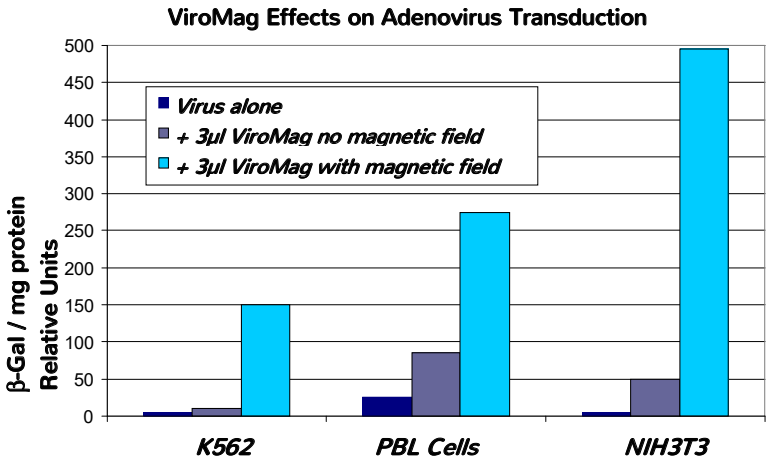
ViroMag is applicable and has been tested successfully on a variety of immortalized cell lines (293-HEK CHO, B95a, HeLa, HT1080, K562, L, NIH3T3, VERO, BT4C...) and primary cells (PAEC, PBL...).

Application examples of magnetic nanoparticles including ViroMag for viral applications

ViroMag increases transduction efficiency

1) ***Adenovirus***

a) The combination of paramagnetic nanoparticles with adenovirus has shown up to 500-fold enhancement of gene expression compared with standard infection [1, 2]. Transduction of suspension cells (K562 and human peripheral blood lymphocytes) has been seen only with magnetic nanoparticules [1-3]. Enhancement of Adenovirus (Ad5 vector) transduction has also been reported on CHO cells [4]. In addition, magnetic field-guided local transduction was demonstrated in vivo (stomach) with an adenovirus combines to magnetic nanoparticles [1].



3 x 10^5 **NIH 3T3 cells** (6 well-plate), 3 x 10^5 **K562 cells** (24 well-plate) and 3 x 10^5 **PBL** (96 well-plate) were infected with of recombinant adenovirus (coding for LacZ) +/- ***ViroMag***.

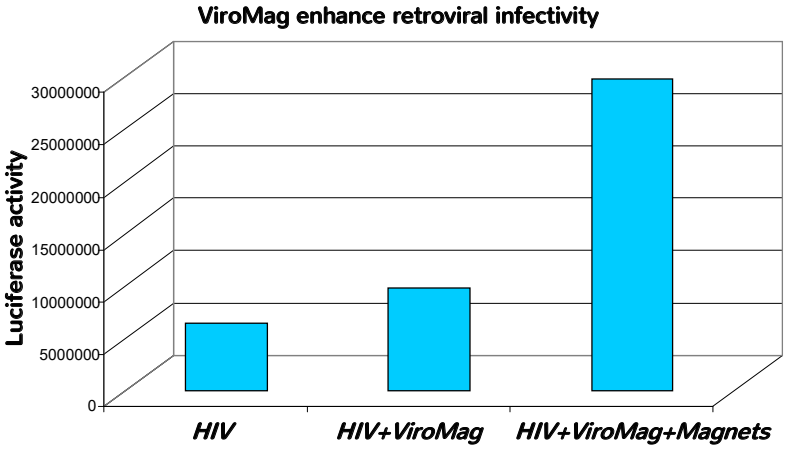
. NIH3T3 were transduced with 200 MOI +/- 3µL ViroMag
. K562 were transduced with 200 MOI +/- 6µL ViroMag
. PBL were transduced with 500 MOI +/- 3µL ViroMag

b) In the same way, *Pandori et al.* have reported that nanoparticles conjugate to Adenovirus significantly enhance their ability to transduce target cells in vitro [5]. This approach required a chemical modification of viral envelopes or viral surface proteins to contain the binding moiety to the magnetic nanoparticles. This lengthy approach and the genetic modification of viral envelopes strategy have shown limited success in many occasions and a more straightforward method is preferable. In contrast, ***ViroMag*** allows you to achieve identical results, in one step procedure to associate virus and paramagnetic nanoparticles, without the requirement of genetically or biochemically modifying your virus.

2) ***Adeno-associated virus (AAV)***. The transduction efficiency of cells infected with AAV bound to magnetic micropsheres has been shown to be 10-fold higher than unbound vectors in HeLa cells [6]. In this report, higher and localized transduction efficiency was also achieved *in vivo* when AAV bounds to magnetic particles were administered either intramuscularly or intravenously.

3) ***Retrovirus / Lentivirus***

a) 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 not at a ratio of 1 µL of ***ViroMag*** per mL of rHIV-Luc supernatant. Mixtures were added to U87-CD4-CCR5 cells and luciferase activity was measured at 72 hours. ***ViroMag*** clearly increased the HIV-1 infection efficiency as shown in figure below.



0.5 x 10^5 U87-CD4-CCR5 cells (96 well-plates) were infected with a recombinant HIV-1-Luc +/- ***ViroMag*** and +/- the magnetic field. Luciferase activity was measured 72h post-transduction.

b) The infectivity of lentiviruses (HIV-1 and a pseudotype lenti-VSVG) has been shown to be increased by about 100-fold when the virus were adsorbed on magnetic nanoparticles [7].

c) A magnetic retroviral vectors formed by the combination of paramagnetic nanoparticles and a Retrovirus (Moloney Leukemia Virus) demonstrated major higher gene transduction efficiency [8].

4) ***Measles Virus***. Kadota, S.I., et al. 2005. *J. Virol. Methods* [10]
Magnetofection enhanced the infection of adenoviruses and retroviruses. It is also shown that Magnetofection enhances the infection of measles virus, a paramyxovirus [10]. When cells expressing a measles virus receptor human SLAM were infected with a measles virus that encodes the green fluorescent protein, Magnetofection enhanced measles virus infection by 30- to 70-fold. The infection of SLAM-negative cells with measles virus was also enhanced by Magnetofection, but to a lesser extent. These results indicate that Magnetofection could be useful for isolation of measles virus from clinical specimens.

5) ***Baculovirus*** *in vitro* transduction efficiency has been significantly increased with magnetic particles [11].

ViroMag concentrates viral dose, promotes and accelerates the infection process

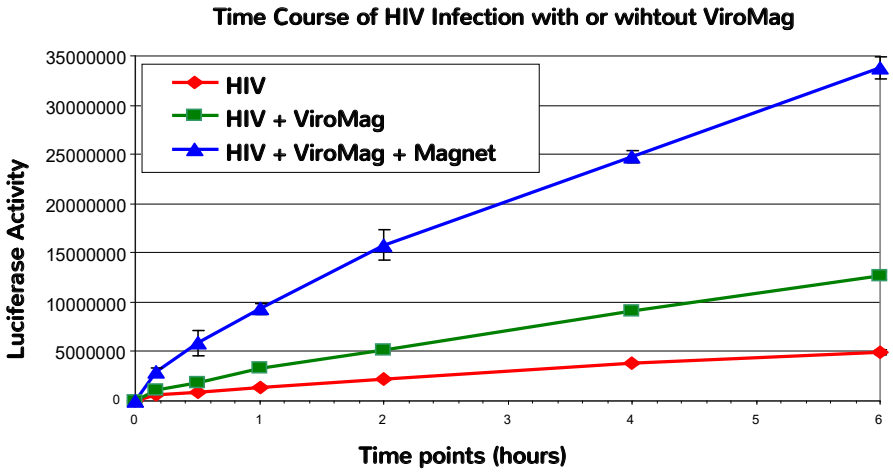
✓ concentrate viral dose and accelerate the infection process [1-12]

1) ***Retrovirus / Lentivirus***.

a) Concentration of viruses from cell culture supernatants has been reported wherein retroviral titers could be increased by 1000 to 4000 fold [9].

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 [7]. Cellular uptake of HIV-1 was increased by 70-fold.

c) Concentration and acceleration of the infection process has also been demonstrated with a pseudo-typed HIV-I virus carrying a luciferase reporter gene. 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 or not with ***ViroMag*** at a ratio of 1 µL of ***ViroMag*** per mL of rHIV-Luc supernatant. Mixtures were added to U87-CD4-CCR5 cells with or without magnetic field. In order to monitor the time course of infection, virus supernatant was washed out after 0.5, 1, 2, 4 and 6 hours and luciferase activity measured at 72 h.



2) ***Adeno-associated virus (AAV)***. 1% of AAV vector bound to magnetic particles resulted in same level of transduction than 100% of the free vector due mainly to their concentration on cell surfaces [6].

✓ Isolate virus from low virus containing samples with ViroMag [10, 12]

1) Concentration of ***measles virus*** [10]. Magnetofection allow the detection of transduction when measles virus stock solution (5×10^2 $TCID_{50}$) was diluted up to 125-fold

2) Non enveloped virus (***SV40***) and enveloped virus such as ***Sindbis virus, HSV type I and VSV*** have also been successfully concentrated with magnetic nanoparticules (up to 100 times for DNA viruses and up to 1000 fold for RNA viruses) to enhance the sensitivity of virus detection by polymerase chain reaction [12]. *Satoh et al.* have reported a reduced infectivity of the viruses associated with magnetic nanoparticles having a size of 0.8µm in diameter and coupled to a very large polymer. This is not surprising since the beads size preclude to internalization and infection and the polymer biological activity is extremely low. In contrast, ***ViroMag*** small nanoparticles formulation is concentrating viruses in the same way and is improving viruses' infectivity as demonstrated for various types of viruses due to their small size and the particularly active polymers associated [1-4, 10, 11].

✓ Magnetic nanoparticles can restore transduction efficiency

Transduction efficiency of PEGylated ***adenovirus*** has been restored by the use of paramagnetic nanoparticles [4]. Polyethylene Glycol (PEG) conjugate to adenoviral capsid can protect the vectors from neutralizing antibodies, vector pharmacokinetics and reduce innate immune response. However, when the adenoviral vector PEGylation provides safe features it also blocks *in vitro* transduction. Association of PEGylated adenovirus with magnetic particles can restore and even increase transduction efficiency in comparison to unmodified adenovirus.

ViroMag improves viral infectious capacity

Retrovirus / Lentivirus

a) Enhancement of infectivity with ViroMag. Low retroviral titer preparation was associated to ViroMag and used to transduce NIH-3T3 cells [1-3]. Whereas no transductions were observed with virus alone, ViroMag led in a 20-fold enhancement over a standard transduction approach consisting of Virus plus polybrene (see figure below).

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 [9].

c) The infectivity of a lentivirus was shown to be clearly increased when associated with paramagnetic nanoparticles in comparison to the virus alone [7]. This enhancement of infectivity was seen even at low MOI.

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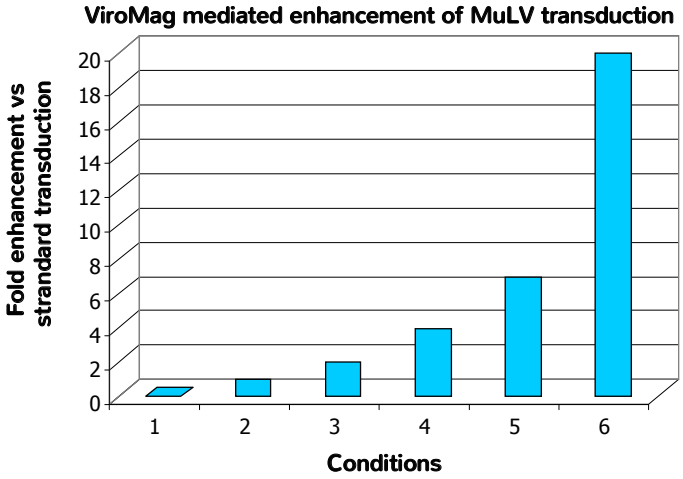


NIH 3T3 cells were infected with a low titer preparation of ***MuLV (Murine Leukemia Virus)*** +/- **ViroMag** in the presence and in the absence of magnetic field. Conditions:

1- Standard Transduction
2- + Polybrene
3- + Polybrene + ***ViroMag*** (no magnetic field)
4- + ***ViroMag*** (no magnetic field)
5- + Polybrene + ***ViroMag*** + magnetic field
6- + ***ViroMag*** + magnetic field

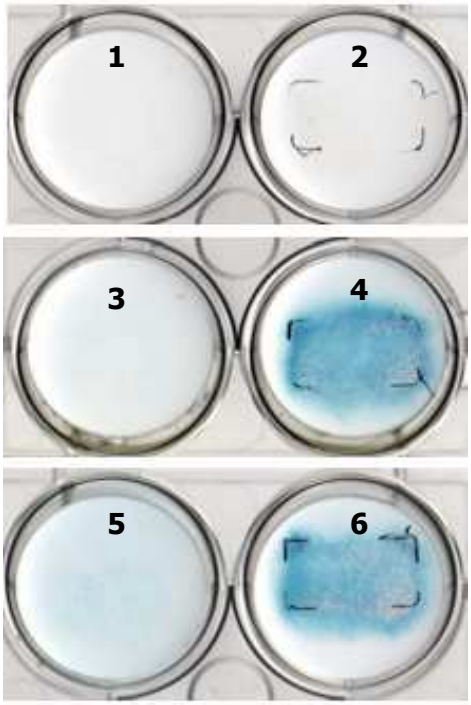
We are grateful to Dr. A. Kruger and C. Plank (Institute of Experimental Oncology, Munich) for kindly sharing these data

ViroMag extends the host tropisms to non permissive cells

1) ***Adenoviruses***

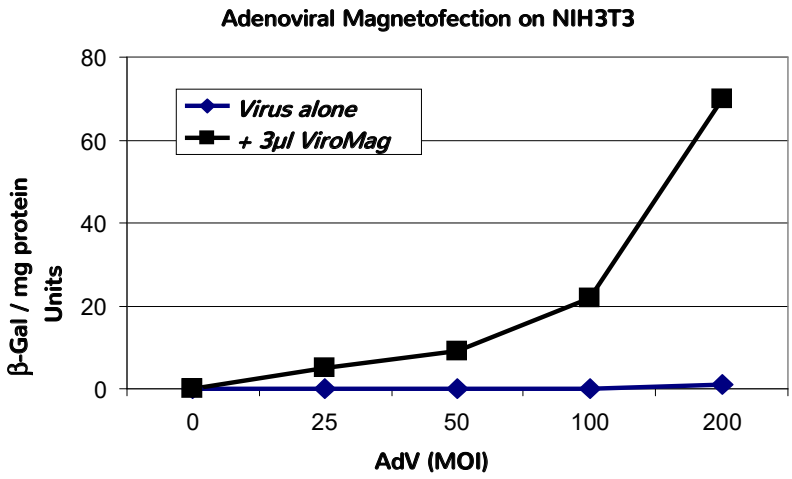
a) Adenoviral infections are dependent on the presence of CAR receptor on the cells surface. Unfortunately, many important and interesting target tissues for fundamental research and gene therapy are non-permissive to viral gene delivery (tumor tissues and apical surface of lung epithelium may express variable, little or none of the required receptors). The association of viral vectors with **ViroMag** is sufficient to force infection of non-permissive cells (lacking CAR) as shown with adenovirus in NIH 3T3, K562 cells and human peripheral blood lymphocytes [1, 2].

Without Magnetic field With Magnetic field



3 x 10^5 **NIH 3T3 cells** (lacking CAR) were infected with a constant dose (200 MOI) of recombinant adenovirus (coding for LacZ) +/- ***ViroMag*** in the presence (right) and in the absence (left) of permanent magnets positioned under the culture plates in a 6 well-plate.

1) Virus alone
2) PBS plus ***ViroMag*** (6µl)
3) & 4) Virus plus ***ViroMag*** (3µl)
5) & 6) Virus plus ***ViroMag*** (6µl)



We are grateful to Dr. C. Plank and Dr. M. Anton (Technical University, Munich) for kindly providing these data.

b) Likewise, *Pandori et al.* have shown that nanoparticles conjugate to ***Adenovirus*** allow transduction of less permissive cell line (C6) with a 20-fold transduction improvement over the free virus [5]. Most importantly as described above, they also demonstrated the possibility to infect cells markedly non-permissive (COLO 205) to adenovirus only after association with nanoparticles. ***ViroMag*** allows you to achieve identical results without the requirement of biochemical modification of your virus.

2) ***Measles virus***. Infection of SLAM-negative cells (VERO, HeLa, CHO and L cells) with the measles virus has been described with very low dose of virus (5 x 10^2 $TCID_{50}$) [10].

ViroMag allows the synchronization of the transduction

Synchronized infection by HIV-1 of primary endothelial cells was reported with the use of magnetic nanoparticles [7]. The number of productive adsorption events by virus alone reached a maximal level after 20h which corresponds to a decrease in residual infectivity left in the culture medium. In contrast, 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.

ViroMag can provide a magnetic targeting

1) A recombinant ***adenovirus*** carrying the *LacZ* gene (200 MOI) was combined with *ViroMag* and incubated on NIH 3T3 cells (lacking CAR) in the presence (left) and in the absence (right) of a magnetic field for 5 minutes. High transduction can be achieved under magnetic influence and can be confined to the area where the magnet has been positioned. Similar results have been published with the same technology [1].

2) In another study, a specific targeting to define area has been shown with a recombinant ***adenovirus*** carrying the *LacZ* gene coupled to microbeads or to paramagnetic microparticles [5]. This paper has used a stylish design to clearly demonstrate the magnetically targeted transduction.

3) Targeting the region of transduction has also been reported with an ***adeno-associated virus*** complexed to magnetic microsphere [6].

4) In the same way, magnetic targeting confine to specific area has been reported with an avidin modified ***Baculovirus*** [11]. ***ViroMag*** allows you to achieve identical results without the requirement of genetically or biochemically changing your virus.

5) In vitro magnetic targeting has also been demonstrated with ***Retrovirus*** [9]. 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) [8].

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Alpha virus, Herpes virus, Polyomavirus (SV40)

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