AdenoMag Results

OZ Biosciences is delighted to announce the launching of a new product based on the Magnetofection ™ technology, specifically designed for ***Adenoviral*** and ***Adeno Associated Viral (AAV)*** applications: **AdenoMag**. 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.

AdenoMag is applicable to all adenoviral and AAV vectors and presents unique properties allowing to:

1. Increase transduction efficiency in terms of percentage of transduced cells and transgene expression
2. Concentrate the entire 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 without modification of the viruses
7. Target/confine transduction to specific area (magnetic targeting)

AdenoMag is the only reagent available offering a solution to such adenoviral applications. **AdenoMag** and adenovirus or AAV to be transduced are mixed in a one-step procedure; no molecular biology process or biochemical modification is required. This reagent demonstrates an exceptionally high efficiency to promote, control and assist ***Adenoviral*** and ***AAV*** transductions.

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

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

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

- ✓ **AdenoMag** specifically optimized for adenoviral and AAV vectors
- ✓ **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 applications
- ✓ **NeuroMag** dedicated transfection reagent for neurons and neuronal cell lines
- ✓ **CombiMag** designed to be associated with all transfection reagents
- ✓ **SilenceMag** created specifically for all siRNA applications.
- ✓ **FluoMag** (fluorescent nanoparticles) to track and analyze delivery (biodistribution, cytometry,...)

Virus Types

AdenoMag reagent can be combined with any adenovirus such as Adenovirus Type 5 (Ad5), Oncolytic Adenovirus (Ad520), AAV serotype 6. It is suitable for any adenoviral- or AAV vector. OZ Biosciences is maintaining an updated list of adenovirus or AAV successfully tested available on the website: www.ozbiosciences.com.

Cell Types

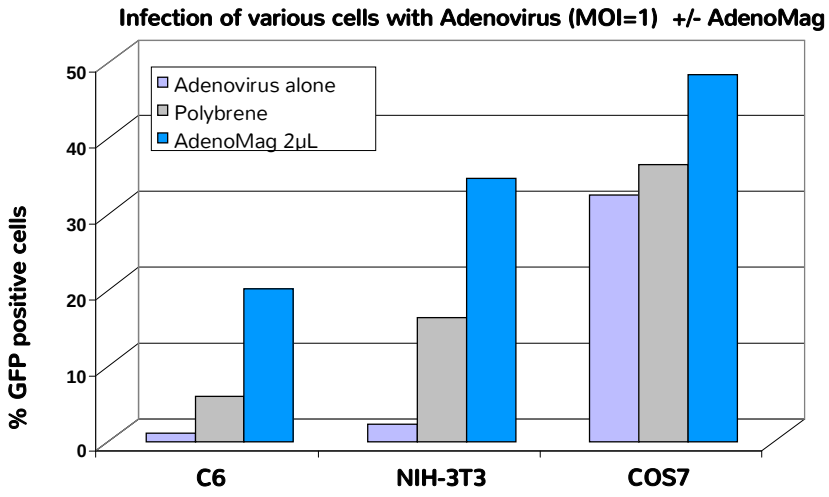
AdenoMag is applicable and has been successfully tested on a variety of cells such as: HEK-293, CHO, HeLa, COS, RAW, NIH3T3, HMEC-1, C6... Please consult our updated list of cells successfully tested available on the website: www.ozbiosciences.com. **AdenoMag** is generally applicable on numerous cell types, but if a particular cell type is not listed, this does not imply that **AdenoMag** is not going to work. OZ Biosciences is going to frequently update this list.

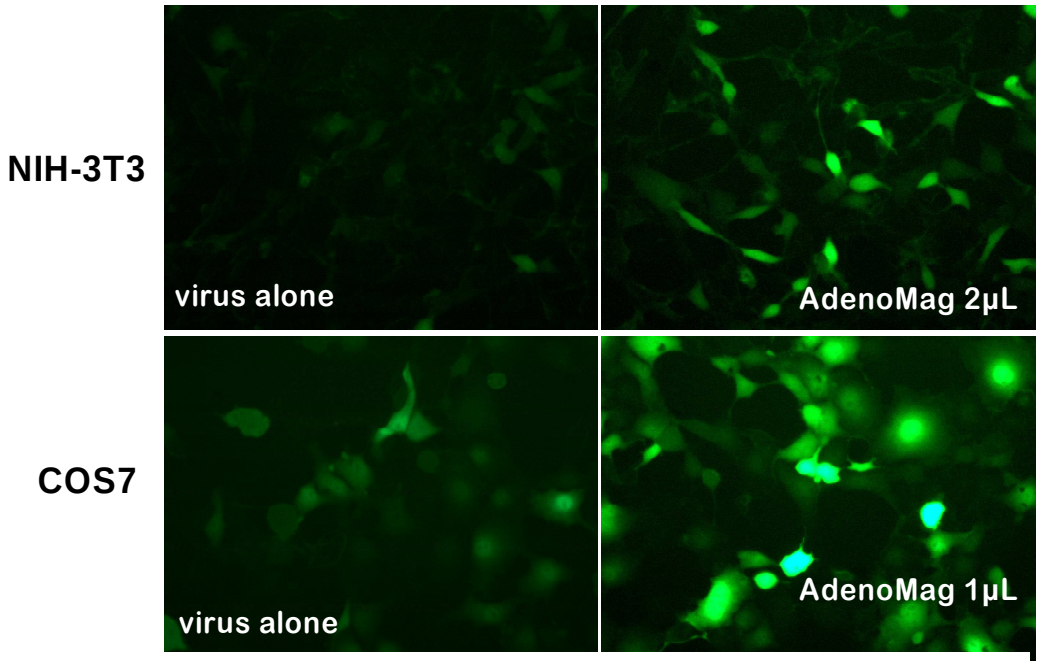
Applications and Results

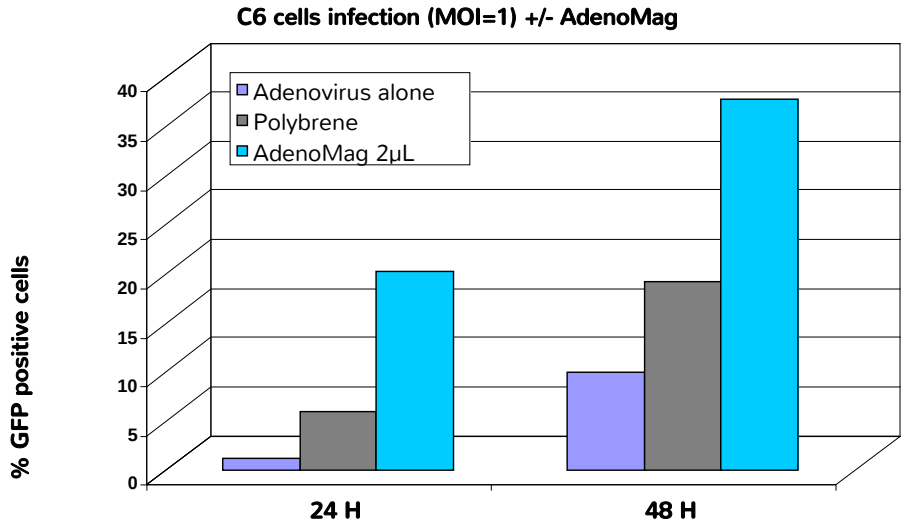
AdenoMag increases transduction efficiency

AdenoMag enhances the number of transduced cells.
Transduction efficiency of an adenovirus carrying a GFP reporter gene –*Ad-CMV-GFP* Adenovirus type 5 (dE1/E3), was assayed in different cell types. 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 8 µg/mL polybrene or in presence of 2µL of AdenoMag. After 25 min of Magnetofection™ (incubation of adenovirus/AdenoMag complexes with cells on a magnetic plate) as indicated in AdenoMag protocol, cells were placed in a 5% CO_2 incubator at 37°C. All cells were analyzed 24 hours post-transduction except the C6 cells that were observed at 24 and 48 h. The number of GFP positive cells (%) and the GFP fluorescence intensity were monitored by FACS.

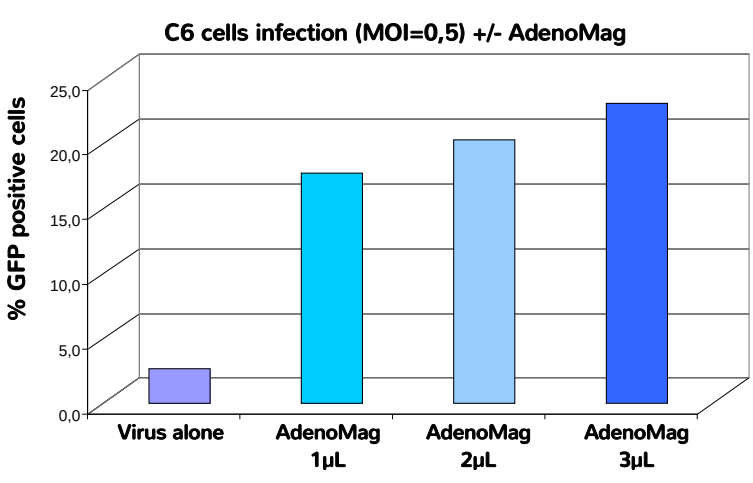
AdenoMag increases infection in different cells at 24h, MOI=1

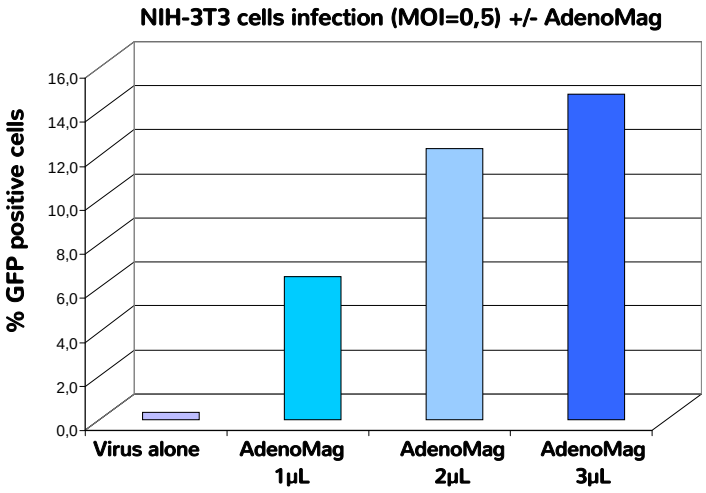


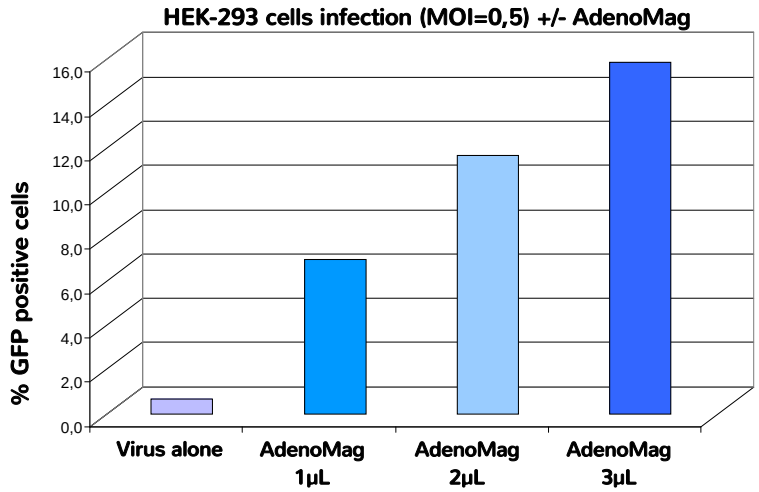




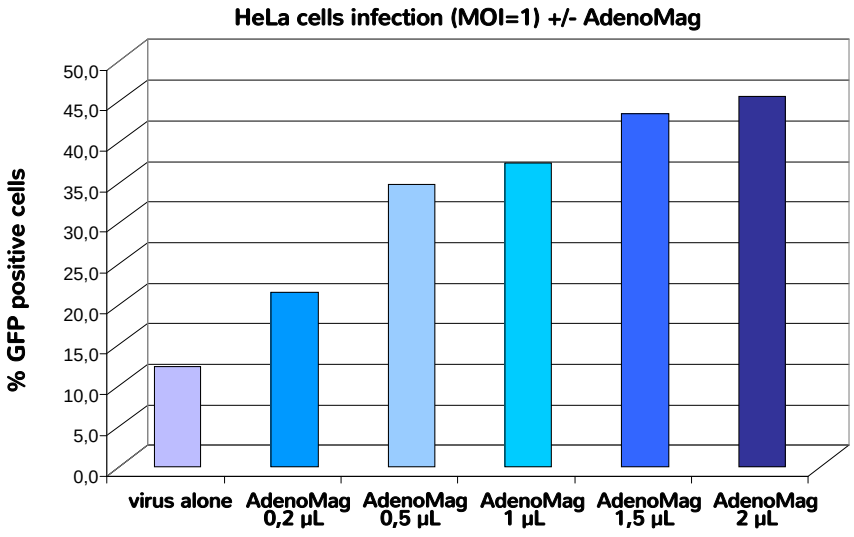
Infection at MOI=0.5 with various doses of AdenoMag in different cells after 24h.

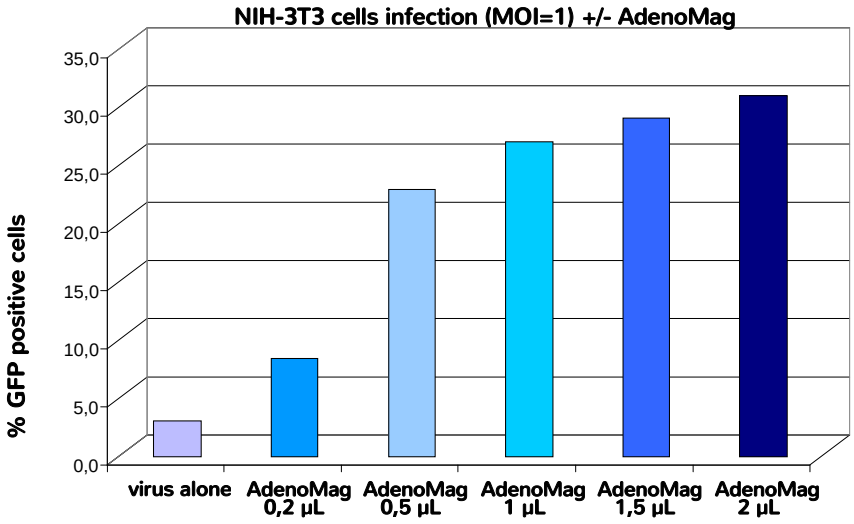


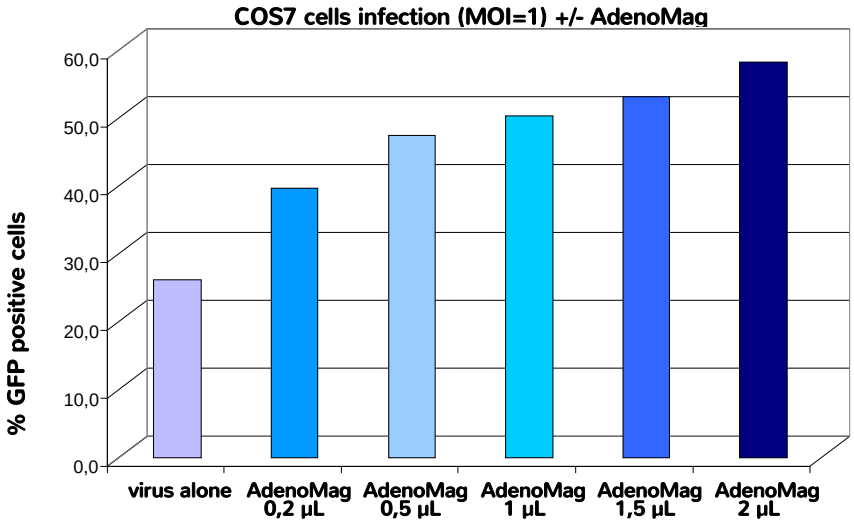




Infection at MOI=1 with various doses of AdenoMag in different cells after 24h.

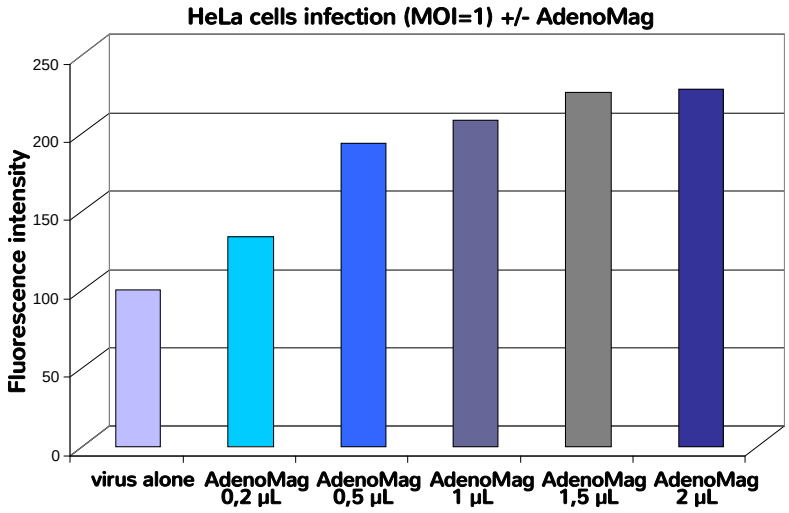


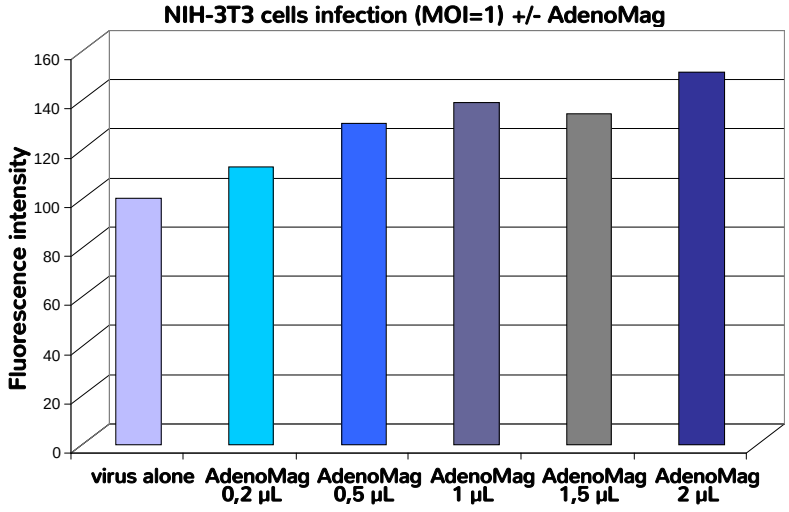


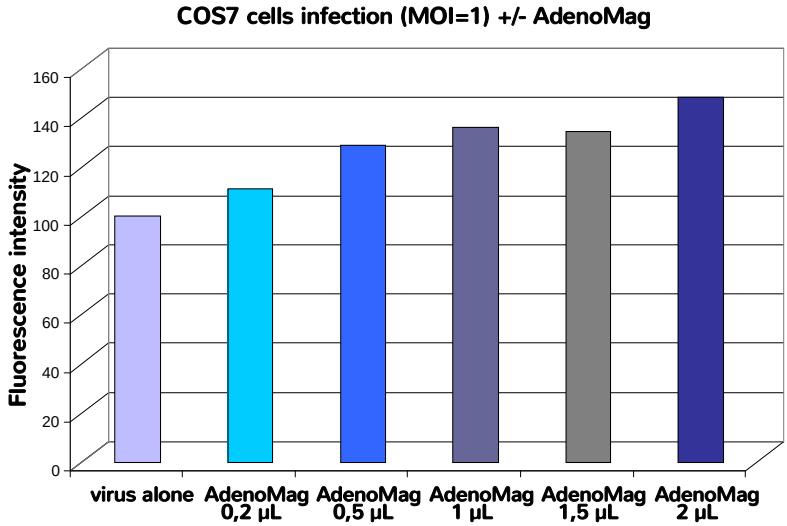


AdenoMag increases level of viral transgene expression

Green Fluorescent

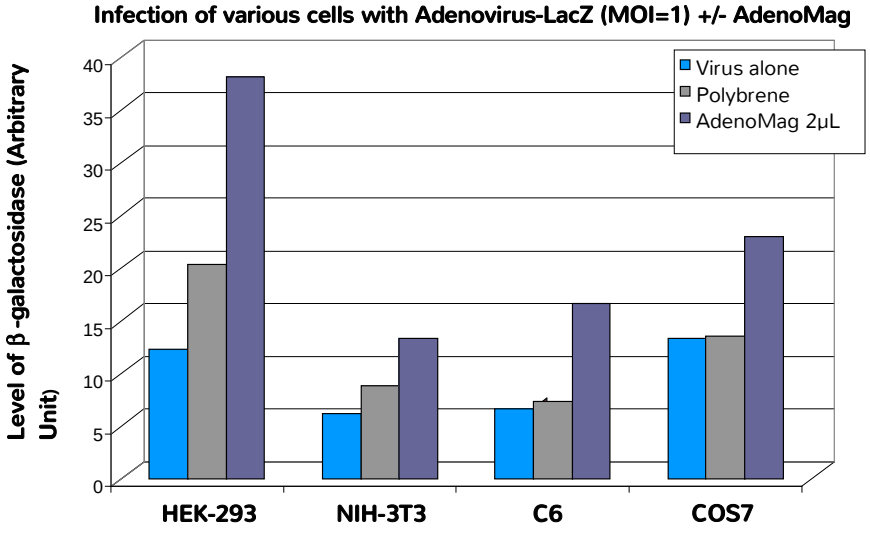


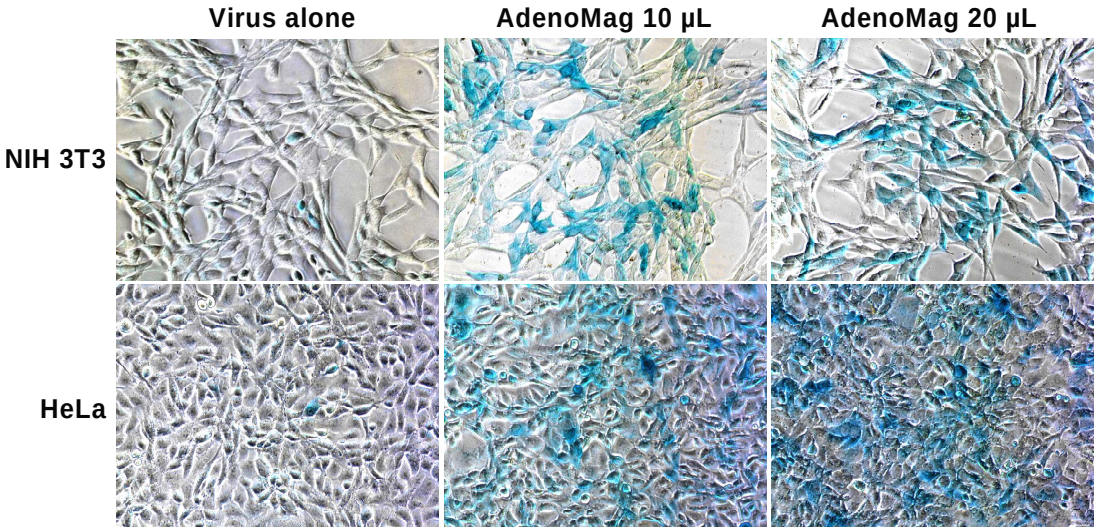




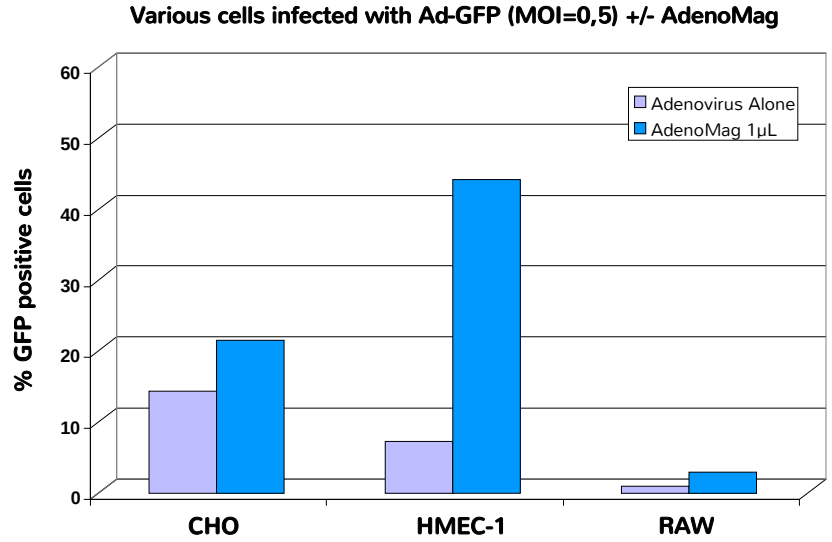
Beta-Galactosidase

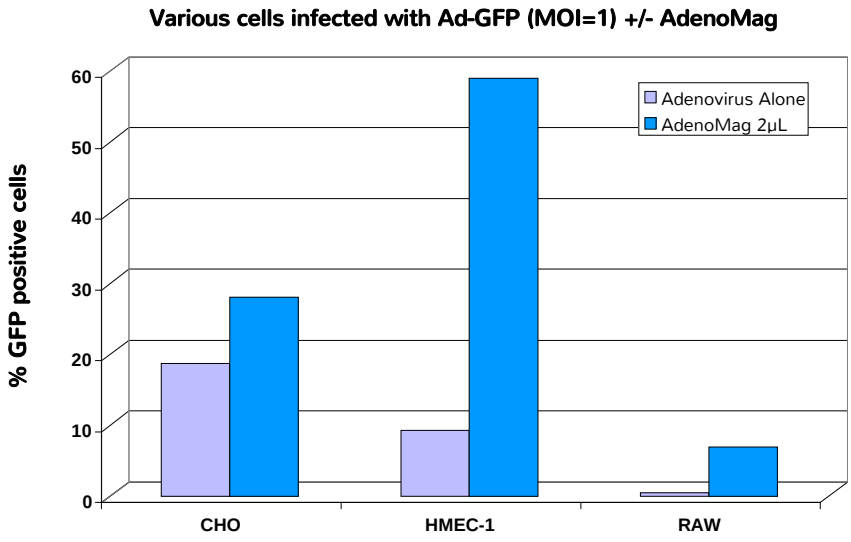
Transduction efficiency of an adenovirus carrying a LacZ reporter gene – *Ad-CMV-LacZ* Adenovirus type 5 (dE1/E3), was assayed in different cell types. Cells were plated the day before infection in a 24-well plate, and were then infected with 1, 5 or 10 MOI either +/- 2, 10 and 20µL of AdenoMag, respectively. Where indicated polybrene at 8µg/mL was used. After 25 min of incubation of adenovirus/AdenoMag complexes with cells on a magnetic plate as indicated in AdenoMag protocol, cells were placed in a 5% CO_2 incubator at 37°C. All cells were analyzed 24 hours post-transduction. Beta-galactosidase expression was then monitored with the -galactosidase CPRG assay kit (OZ Biosciences, catalog # GC10002) and X-Gal staining kit (OZ Biosciences, catalog # GX10003).





AdenoMag is perfect for hard-to-infect cells



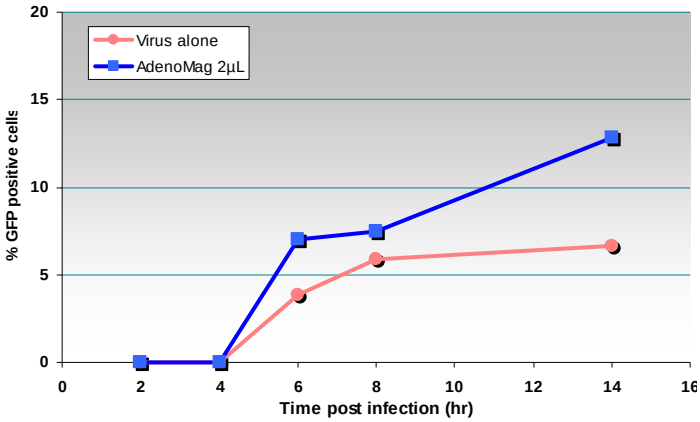


AdenoMag concentrates viral dose, promotes and accelerates the infection process

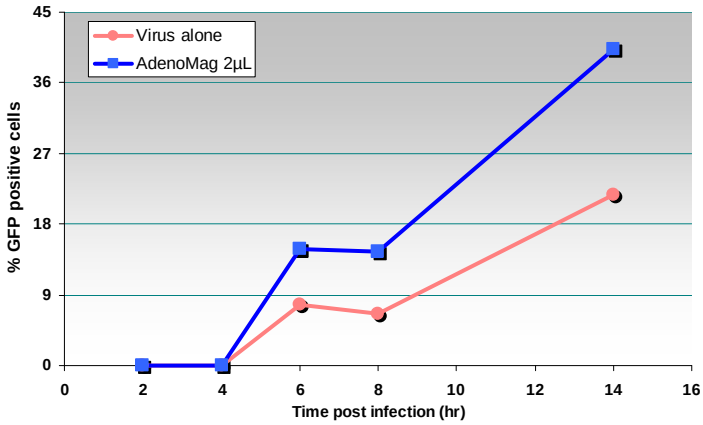
1) ***AdenoMag accelerates the infection process in terms of infectivity***

Transduction efficiency of Ad-GFP was assayed in C6, COS7, NIH-3T3 and HEK-293T cells. Cells were plated the day before infection in a 24-well plate, and were then infected with a MOI=1 in presence or not of 2 µL AdenoMag. After 30 min of Magnetofection™ (incubation of adenovirus/AdenoMag complexes with cells on a magnetic plate) as indicated in AdenoMag protocol, cells were placed in a 5% CO_2 incubator at 37°C. This time was set as time 0 for the kinetics. % GFP+ cells were determined at time 2, 4, 6, 8 and 14 h post infection by FACS measurements. All cell type showed an increase in GFP+ cells and in transgene expression as soon as 6

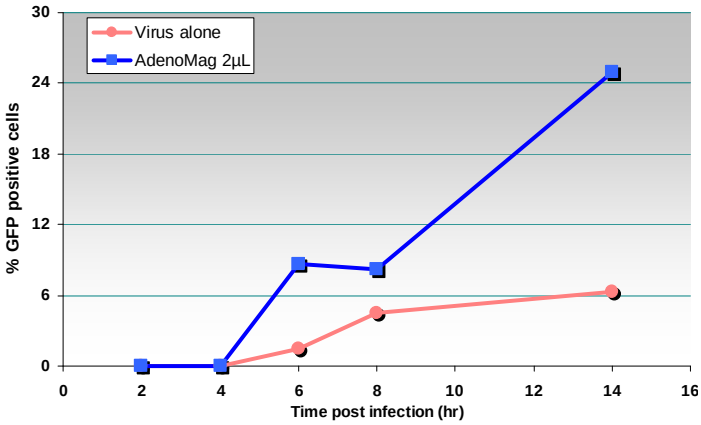
C6 cells infection kinetics (MOI=1) +/- AdenoMag



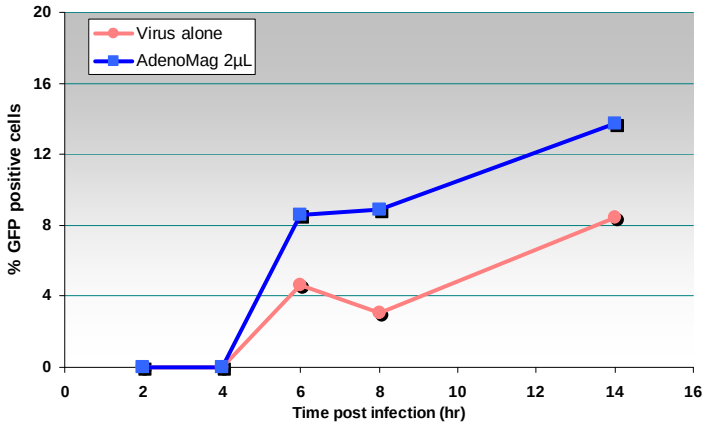
COS7 cells infection kinetics (MOI=1) +/- AdenoMag



NIH-3T3 cells infection kinetics (MOI=1) +/- AdenoMag

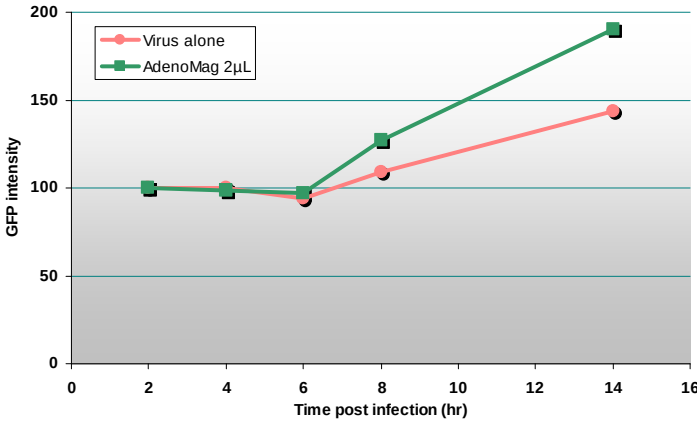


HEK-293T cells infection kinetics (MOI=1) +/- AdenoMag

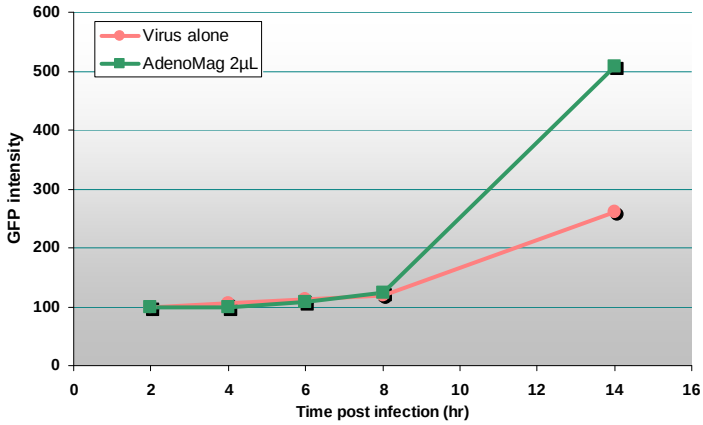


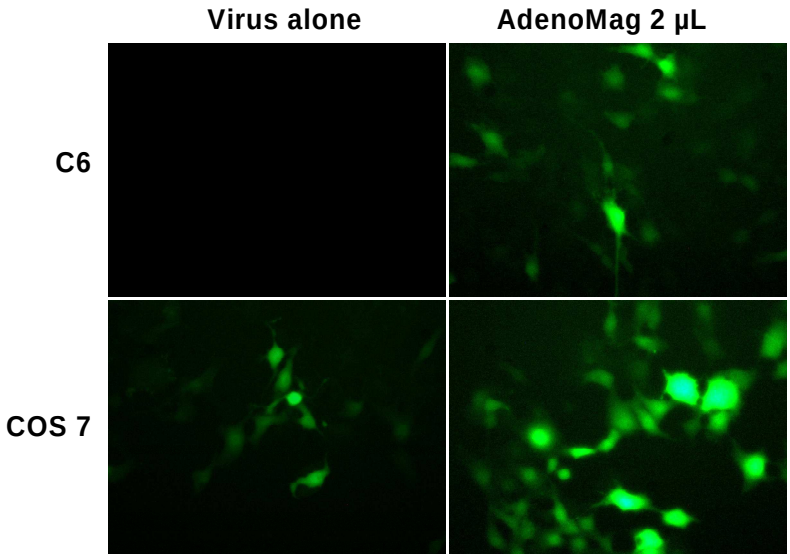
2) ***AdenoMag accelerates the infection process in terms of transgene expression***

C6 cells infection kinetics (MOI=1) +/- AdenoMag



COS7 cells infection kinetics (MOI=1) +/- AdenoMag



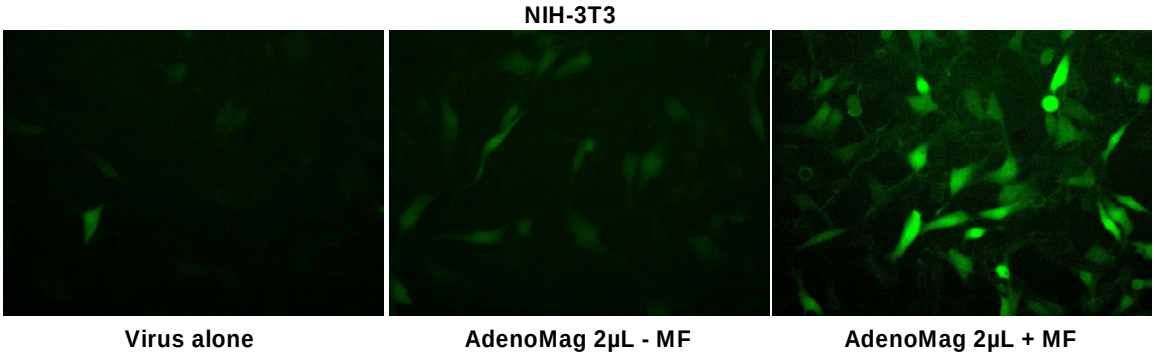


Photos taken at the end point of the kinetics (14 hours post infection)

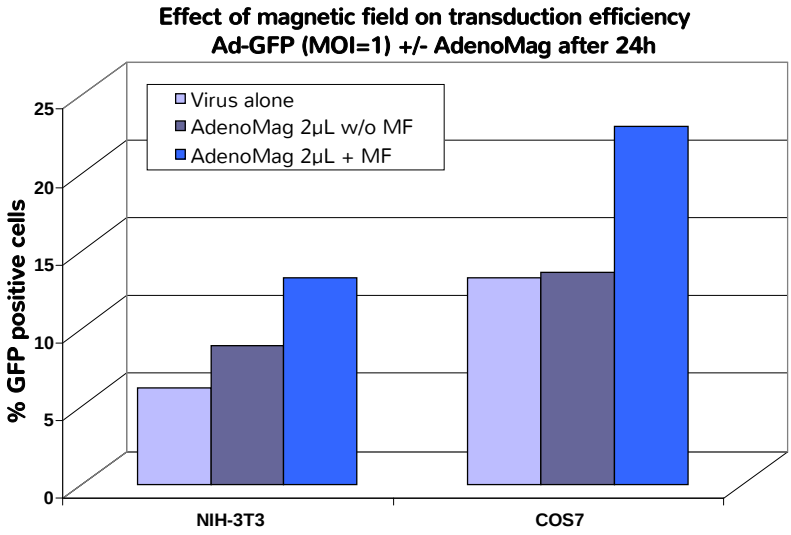
3) ***AdenoMag rapidly concentrates Adenovirus onto cells as demonstrated by magnetic field effect and washing procedure.***

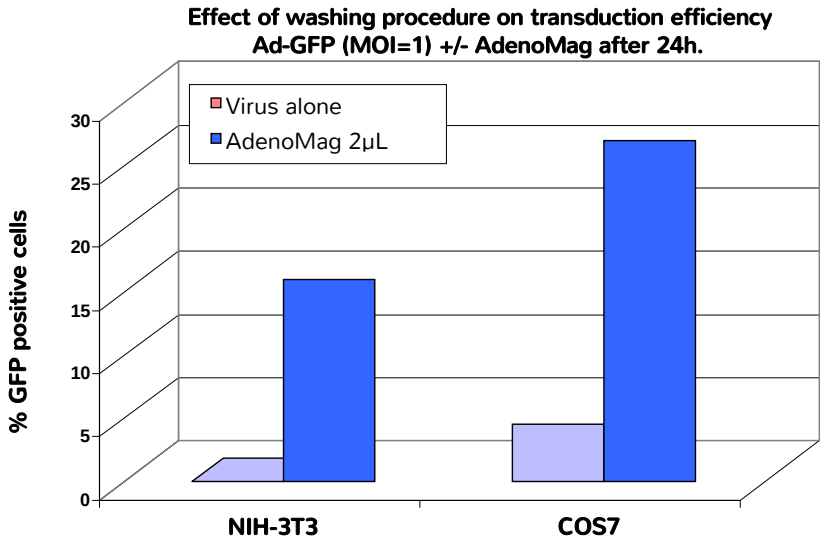
(**A**) NIH-3T3 and COS7 cells were infected with Ad-GFP at MOI of 1 +/- 2µL of AdenoMag. After the adenovirus/AdenoMag complex formation cells were infected with adenovirus alone, or with adenovirus/AdenoMag complexes and placed (+ MF) or not (- MF) on the Magnetic plate. Fluorescent cells were detected 24h post infection by FACS and microscopy. (**B**) Cells were infected as above except that after 30 min of incubation on the magnetic plate, cells were immediately washed as described in AdenoMag protocol. GFP positive cells were analyzed 24h later by FACS.

(A)



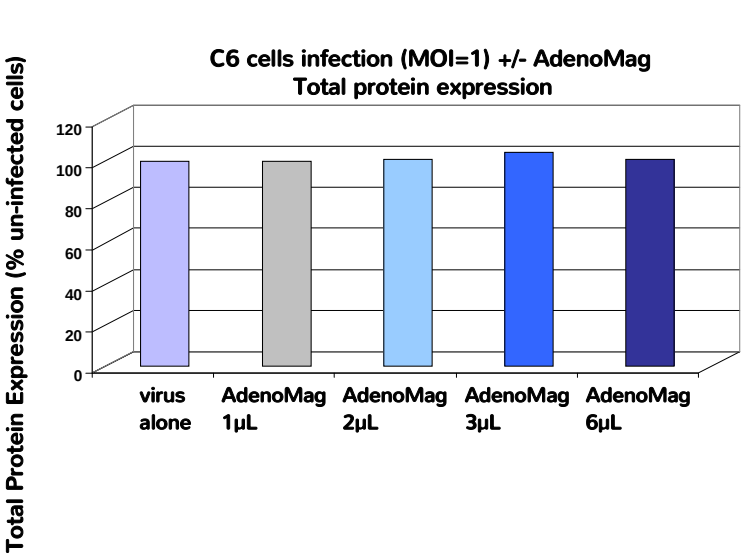
(B)

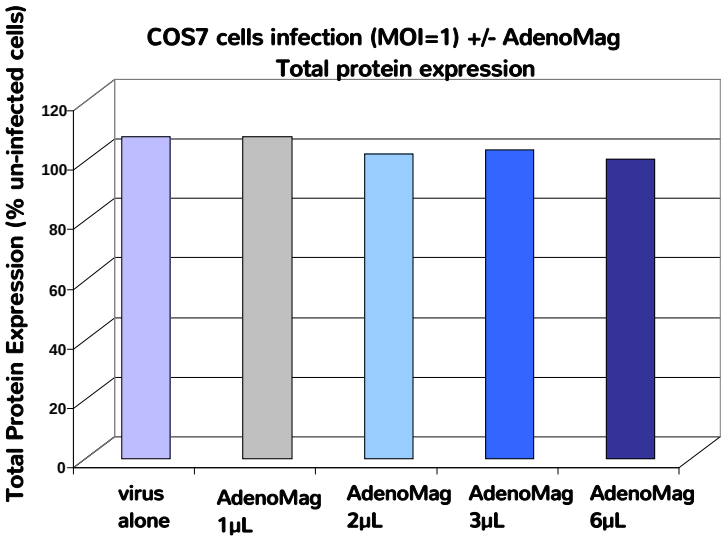


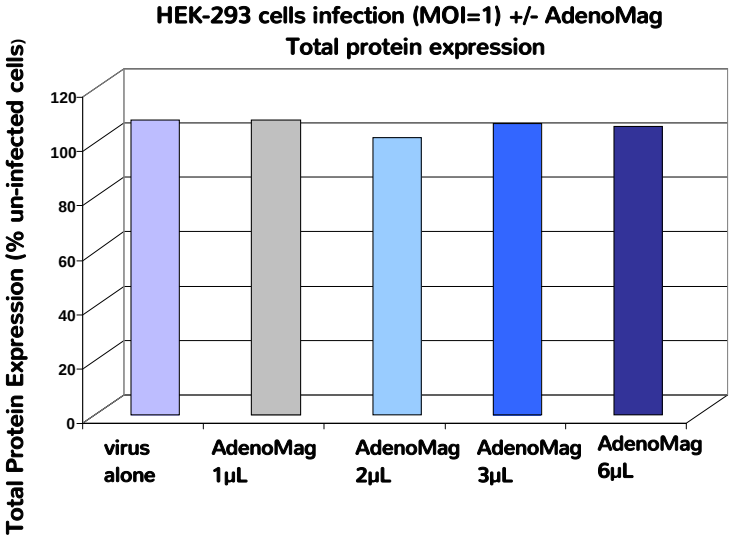


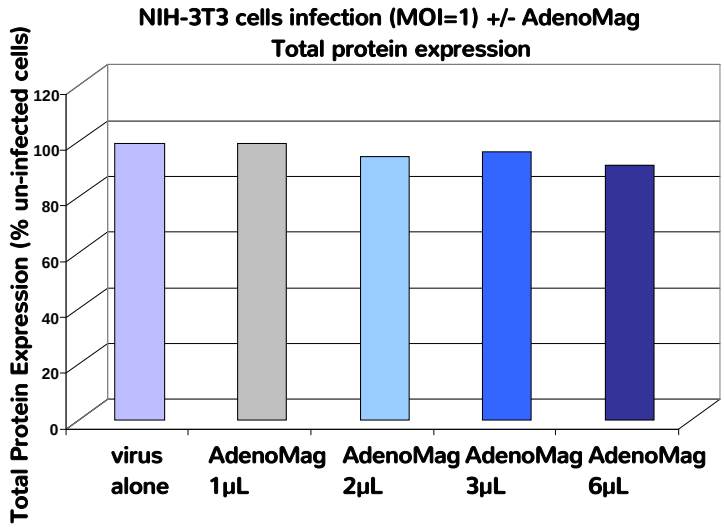
Effect of AdenoMag and infection on cell viability

Cells were infected with Ad-GFP or Ad-LacZ +/- AdenoMag at a MOI of 1 for 24 h. Total protein expression was determined with Bradford assay kit (OZ Biosciences cat# BA00100). Results show the % of total protein expression related to un-infected cells.









Increase Oncolytic adenovirus effect with AdenoMag

Effect of Ad520 oncolytic activity +/- AdenoMag in CAR-deficient multidrug-resistant 181RDB-fLuc cells [1, 2]. Cells were infected with Ad520 +/- AdenoMag in cell culture medium containing 7.5% FCS with (+ MF) or without (- MF) positioning on the Magnetic plate for 30 min after adding virus or magnetic complexes. 24 h post-infection, the virus-containing medium was replaced with fresh cell culture medium.

- Effect on the MOI: The AdenoMag / Ad520 complexes under magnetic field increased oncolytic effect at low MOI in comparison to adenovirus alone or AdenoMag / Ad520 complexes without MF. IC50 is achieved with a MOI of 5 in presence of AdenoMag whereas with virus alone IC50 is obtained with a MOI of 100.
- Effect on the Adenovirus kinetics: AdenoMag allowed reducing significantly the kinetics of oncolytic effect. An oncolytic effect is observed as soon as 2 days after infection when AdenoMag is used whereas 6 days are necessary when the virus is used alone.
- Effect on viral particle production and viral DNA replication: AdenoMag / Ad520 enhanced the production of viral particles when compared to adenovirus alone in CAR deficient 181RDB cells.
- Magnetic targeting. The oncolytic effect obtained with Ad520/AdenoMag complexes under magnetic field can be confined to specific area delimited to magnetic size and shape.

1. Tresilwised N, Mykhaylyk O, Anton M, et al. Tuning of oncolytic adenovirus magnetic complexes: Tumor-killing effect on CAR-deficient multidrug-resistant cancer cells. *Human Gene Therapy.* 2008; 19 (10): 1163.
2. Tresilwised N, Mykhaylyk O, Anton M, et al. Boosting Oncolytic Adenovirus by Magnetic Force. *Molecular Therapy.* 2008; 16 (S1): S176.

Examples of Adenoviral applications with Magnetic nanoparticles [1- 13]

✓ **increases transduction efficiency**

The combination of super paramagnetic nanoparticles with adenovirus has shown up to 500-fold enhancement of gene expression compared with standard infection.

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

Concentration of AAV has been reported. Transduction efficiency of PEGylated adenovirus can be restored by the use of magnetic nanoparticles.

✓ **improves viral infectious capacity**

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

✓ **extends the host tropisms of viral vectors to non-permissive cells**

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.

✓ **can provide a magnetic targeting**

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

1. 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.
2. Mah, C., et al. Improved Method of Recombinant AAV2 Delivery for Systemic Targeted Gene Therapy. *Mol. Ther.* 2002; 6(1): 106-112.
3. Pandori M.W., et al. Adenovirus-Microbead Conjugates Prossess Enhanced Infectivity: A New Strategy to Localized Gene Delivery. *Virology.* 2002; 299: 204-212.
4. Plank C., et al. Enhancing and targeting nucleic acid delivery by magnetic force. *Exp Opin Biol Ther.* 2003; 3(5):745-58.
5. Campos SK., et al. Avidin-based targeting and purification of a protein IX-modified, metabolically biotinylated Adenoviral vector. *Mol. Ther.* 2004; 9(6): 942-54.
6. Schillinger U., et al. Advances in Magnetofection – magnetically guided nucleic acid delivery. *J. Magn. Magn. Mat.* 2005; 293: 501-508.
7. Mok H., et al. Evaluation of polyethylene glycol modification of first-generation and helper-dependent adenoviral vectors to reduce innate immune responses. *Mol. Ther.* 2005; 11(1): 66-79.
8. Bhattarai SR., Et al. N-hexanoyl chitosan-stabilized magnetic nanoparticles: enhancement of adenoviral-mediated gene expression both in vitro and in vivo. *Nanomedicine.* 2008; 4(2):146-54.
9. Kamei K., et al. Direct cell entry of gold/iron-oxide magnetic nanoparticles in adenovirus mediated gene delivery. *Biomaterials.* 2009; (30) 1809-14.
10. Gliddon BL., et al. Isolation, culture and adenoviral transduction of parietal cells from mouse gastric mucosa. *Biomed. Mater.* 2008; (3): doi:10.1088/1748-6041/3/3/034117.
11. Holzbach T., et al. Non-viral $VEGF_{165}$ Gene Therapy – Magnetofection of acoustivally active magnetic microspheres (Magnetobubbles) increases survival in a skin flap model. *J. Cell. Mol. Med.* 2008; epub.
12. Tresilwised N, et al. Tuning of oncolytic adenovirus magnetic complexes: Tumor-killing effect on CAR-deficient multidrug-resistant cancer cells. *Human Gene Therapy.* 2008; 19 (10): 1163.
13. Tresilwised N, et al. Boosting Oncolytic Adenovirus by Magnetic Force. *Molecular Therapy.* 2008; 16 (S1): S176.