Mag4C-Lv Kit - Results

Mag4C-Lv Kit

Magnetic capture for viral concentration & storage buffer for superior viral preservation

OZ Biosciences is delighted to announce the launching of a new product ***Mag4C-Lv Kit,*** specifically designed and developed for capturing, concentrating and storing Lentiviruses and Retroviruses. This kit is composed of 3 reagents allowing **Magnetic Capture/Concentration, Elution and Conservation** of Lentiviruses/Retroviruses and a multipurpose **Magnetic Separation Rack**.

Mag4C-Lv magnetic nanoparticles capture by electrostatic and hydrophobic interactions viruses in culture medium with 80-99 % efficiency. Once captured onto magnetic beads, viruses can be:

(1) Concentrated and stored with the **Conservation Buffer** or directly used for cell culture, molecular biology or other assays.
(2) Concentrated, eluted from the magnetic beads with the **Elution Buffer** and stored with the **Conservation Buffer** or used for various assays.

The **Conservation Buffer** has been expressly designed to improve the stability of Lentiviruses/Retroviruses upon storage conditions. This buffer is fully compatible with magnetic nanoparticles, meaning that virus bound to magnetic beads can be diluted directly into the buffer for long term storage.

Mag4C-Lv Kit is dedicated to Lentiviruses/Retroviruses and presents unique properties:

For concentration

1. Concentration of viruses by magnetic capture in 30-45 minutes
2. Simple, rapid & ready-to-use: No need to process magnetic beads before capture
3. High yield of viral capture and recovery
4. Fast and adjustable concentration (2 to 1000 X)
5. Avoid ultracentrifugation, precipitation and chemicals: no stringent buffer or physical action on viruses
6. Reduced handling steps of viruses (minimized bio-hazard)
7. Suitable for large volume
8. Serum compatible & Non toxic
9. Ideal for cell culture transduction/infection (Magnetofection™ advantages)

For conservation

1. Improved virus preservation upon storage (-80°C)
2. Maintain high virus titers upon freeze and thaw cycles
3. Compatible with magnetic nanoparticles

Virus Types

Mag4C-Lv beads can be combined with any Lentiviruses/Retroviruses.

Downstream Biological Assays

After magnetic capture and concentration, viruses can be used for multiple assays. For instance, they can be used for PCR, western blot, ELISA, *in vitro* and *in vivo* infection, etc. Viruses can be directly used with the bound Mag4C-Lv beads or eluted from the Mag4C-Lv beads (free of beads). For cell biology, we suggest to use viruses associated with Mag4C-Lv beads to infect cells. Virus complexed to Mag4C-Lv beads, eluted virus, and virus in conservation buffer have been successfully tested on a variety of immortalized and primary cells.

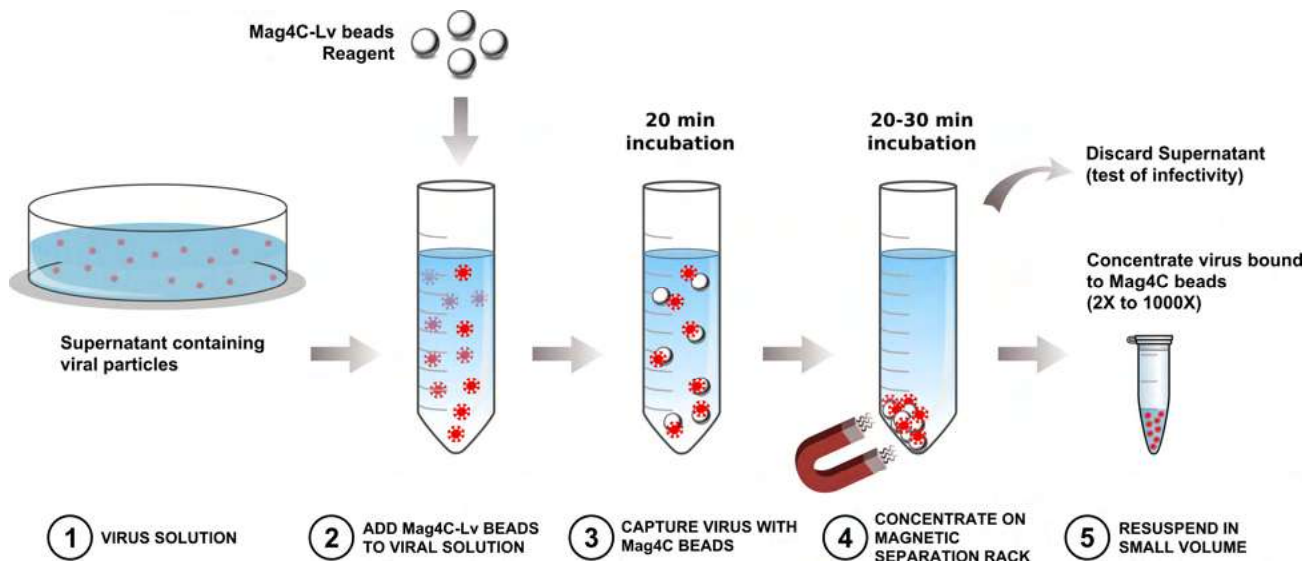
For in vitro and in vivo infection. Mag4C-Lv beads are compatible with the Magnetofection™ technology. This method allows concentrating the entire viral dose on the cells very rapidly, accelerating the transduction process and infecting non-permissive cells. Moreover, virus infection efficiency is significantly increased and cell adsorption/infection can be synchronized without modification of the viruses. Targeted/confined transduction to specific area (magnetic targeting) can also be accomplished.

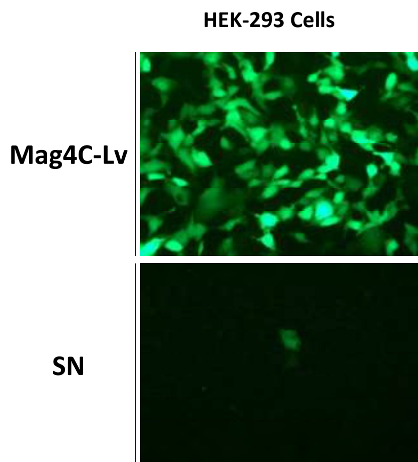
Applications and Results

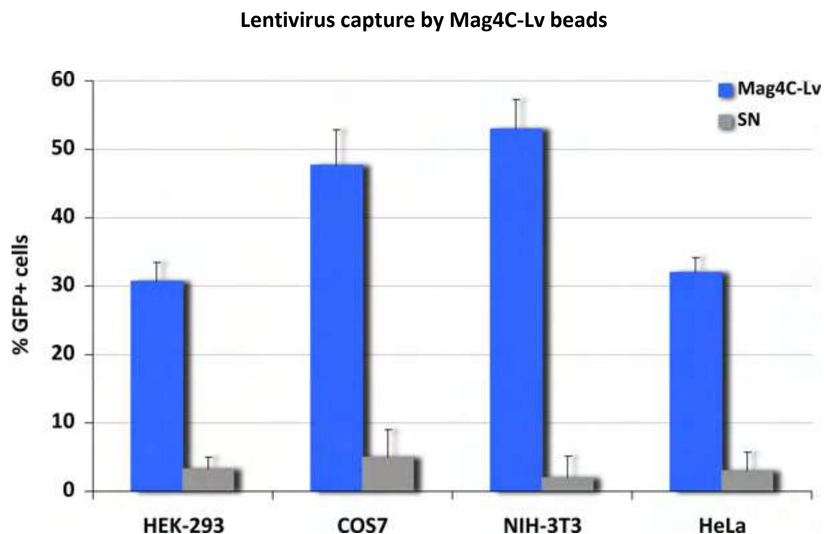
1- Capture and Concentration of Virus with Mag4C-Lv beads

<u>**Mag4C-Lv beads allow capturing and concentrating lentiviruses**</u>

Virus solution containing 10^7 viral particles/mL of HIV-SFFV-GFP was mixed with Mag4C-Lv beads for capture and concentration procedures. Viruses captured by Mag4C-Lv magnetic nanoparticles were concentrated by 15-30 min incubation on the Magnetic Separation Rack. The viruses bound to the Mag4C-Lv beads were retained by the magnetic field, the supernatant was removed and the pellet was re-suspended in a smaller volume in order to concentrate the solution (2x to 100x). Both supernatant (SN) and re-suspended magnetized virus (Mag4C-Lv) were tested for infectivity on HEK-293, COS7, NIH-3T3 and HeLa cells. Cells were analyzed 24h post-transduction and the number of GFP positive cells (%) was monitored by flow cytometry. Refer to the general protocol for detailed information on capture and concentration procedure.



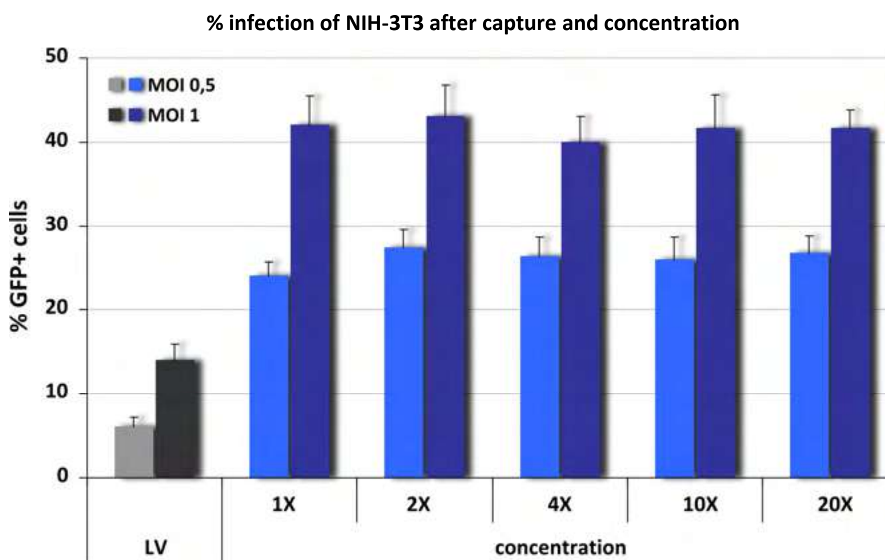


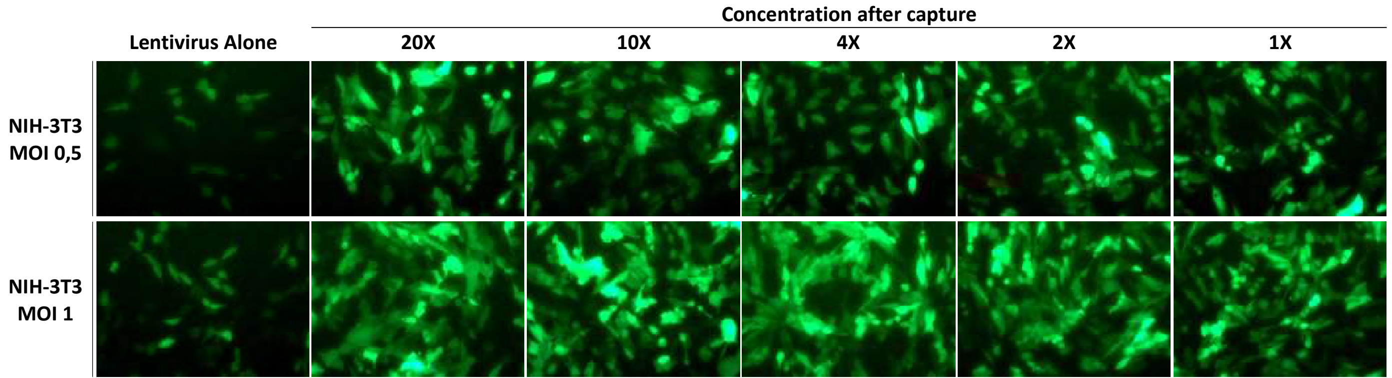


Results show that the Mag4C-Lv beads efficiently capture lentiviruses/retroviruses (Mag4C-Lv, blue bars): supernatants (grey bars) are nearly non infectious (absence of virus) whereas the concentrated virus bound to the beads are highly infectious.

Mag4C-Lv beads allow concentrating virus from 2x up to 1000x

100µL of a virus solution were captured as described above. After discarding the supernatant, lentiviruses complexed to Mag4C-Lv beads were re-suspended in 5 to 100 µL of PBS which correspond to a 1X to 20x concentration. NIH-3T3 and COS7 cells were then infected with virus alone (original solution; Lv control) or with the concentrated magnetized lentivirus at two MOI (0.5 or 1). The % of GFP positive cells were determined 24h by flow cytometry and fluorescence microscopy.



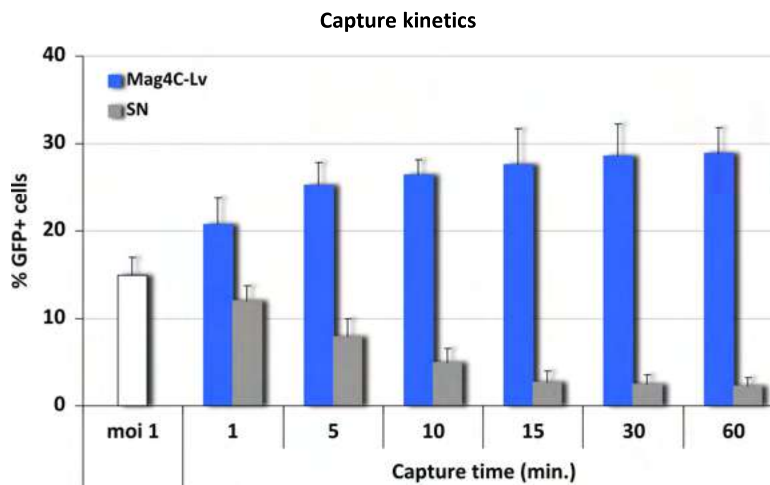


Results show that after capture, lentivirus complexed to Mag4C-Lv beads can be concentrated from 1x to 20x without losing transduction efficiency. Moreover, *in vitro* infection experiments confirm that magnetic nanoparticles associated with the viruses improve transduction efficiency when a magnetic field is applied

Mag4C-Lv beads allow a rapid kinetics of capture.

Mag4C-Lv beads allow capturing lentiviral particles within minutes. 100 µL of a HIV-SFFV-GFP Lentivirus solution (10^7 infectious virus/mL) was mixed with 20 µL of Mag4C-Lv beads and incubated during various times (1, 5, 10, 15, 30 and 60 min) at room temperature (RT). Complexes were then concentrated on the Magnetic Separation Rack during 20 min at RT. Supernatants (SN) were saved and pellets of magnetized virus (Mag4C-Lv) were re-suspended in PBS (20µL, 5X concentration) before assaying their infectivity on HEK-293 cells. % of GFP positive cells was determined 48h by fluorescence (HEK-293) and FACS (HEK-293, NIH-3T3 and COS7 cells).

Results show that viruses are efficiently captured within minutes by Mag4C-Lv beads and that optimal capture efficiency is reached after 15 min.



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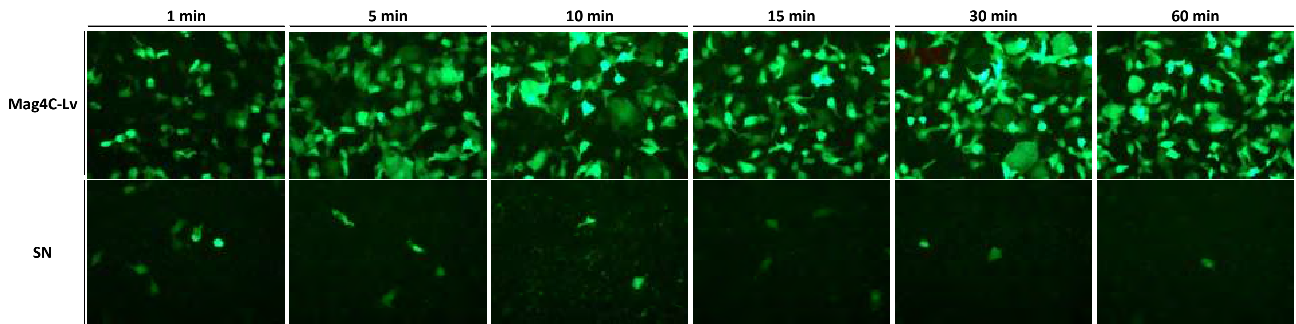
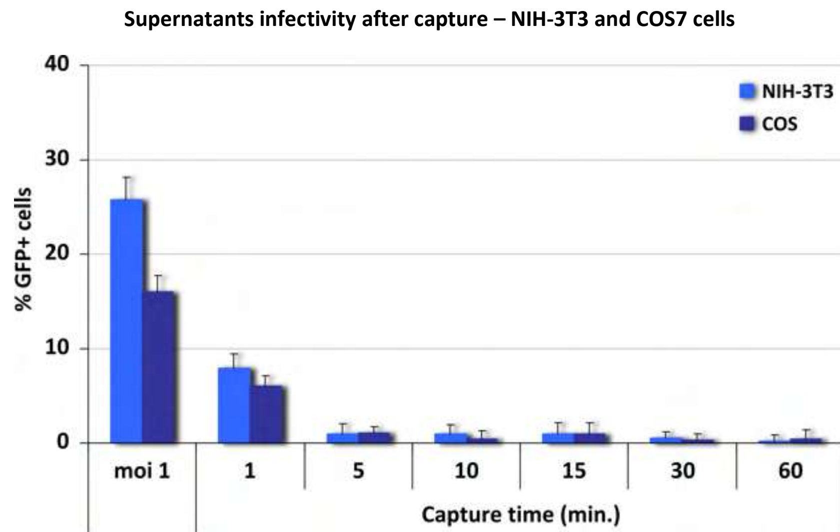
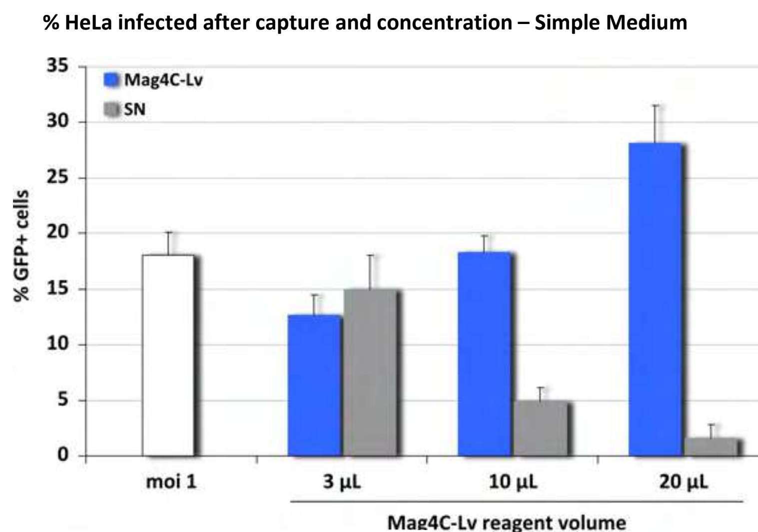




Capture efficiency is medium composition dependent

Simple Medium is defined as medium without serum or complement or with reduced serum conditions (< 5%); for example DMEM, MEM, PBS, etc. HIV-SFFV-GFP (10^6) lentivirus suspended in serum-free and complement-free DMEM were mixed with different volumes of Mag4C-Lv beads (3, 10 and 20 µL). After capture and concentration by the Mag4C-Lv beads as described above, supernatants (SN) were saved and pellets of magnetized virus (Mag4C-Lv) were re-suspended in PBS before testing their infectivity on HeLa cells. % of GFP positive cells was determined after 24 hours by flow cytometry.

Results show that capture is efficient in simple medium and only 10 µL of Mag4C-Lv beads are required to bind 10^6 lentiviral particles. Moreover Magnetofection greatly improves the transduction capacity of the lentivirus.



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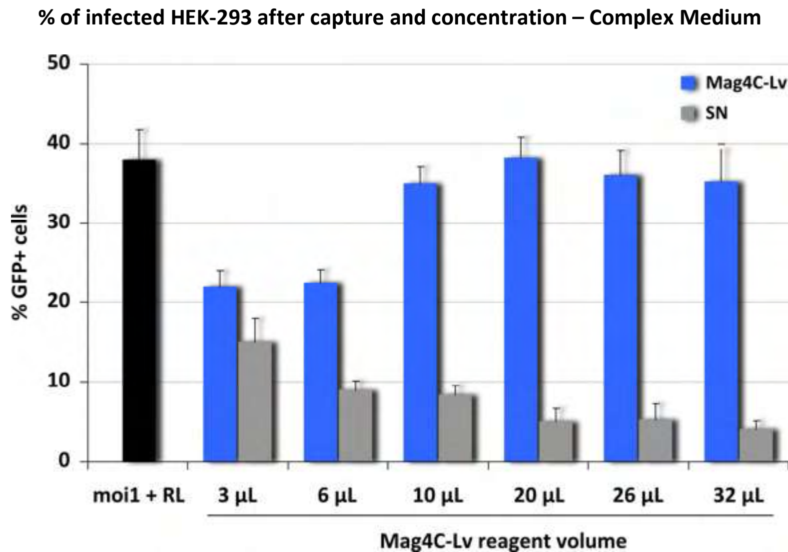
Complex Medium is defined as complete growth culture medium containing serum (≥ 10% serum) and complements. 10^6 viruses suspended in complete DMEM (10% FBS, glutamine, non essentials amino acids, penicillin / streptomycin, sodium pyruvate) were mixed with different volumes of Mag4C-Lv beads (from 3 to 32 µL) and incubated 15 min at RT. After a 15-min concentration step onto the Magnetic Separation Rack, supernatants (SN) were saved and pellets of magnetized virus (Mag4C-Lv) were re-suspended in PBS before testing their infectivity on HEK-293 cells. % of GFP positive cells was determined after 24h by flow cytometry and fluorescence microscopy.



Results show that Mag4C-Lv efficiently capture viruses in complex medium. Moreover, this demonstrates that the quantity of Mag4C-Lv beads to use must be optimized according to the medium composition. In complex medium, Mag4C-Lv beads volume should be raised because capture efficiency of the virus will be slightly hampered due to the presence of competing proteins for the binding to the magnetic particles.

In summary, the results clearly demonstrate the ability of Mag4C-Lv beads to:

1. Efficiently capture Lentivirus/Retrovirus.
2. Concentrate the viruses up to hundred or even thousand times.
3. Capture and concentrate very rapidly
4. Capture and concentrate viruses from a variety of medium (simple and complex)
5. Improve the transduction efficiency (Magnetofection™)

2- Advantages of keeping the Mag4C-Lv beads associated with the viruses

After capture & concentration, Mag4C-Lv beads bound to lentiviruses/retroviruses can be removed by elution (see below) or kept for storage or downstream assays. Keeping the Mag4C-Lv beads bound to lentiviruses/retroviruses offers several advantages:

- For molecular biology. The presence of Mag4C-Lv beads allows changing the medium or buffer easily by using the Magnetic Separation Rack, and thus to adjust the conditions to the assay requirement. The presence of magnetic particles does not affect PCR, ELISA or western blot. Non enveloped virus (*SV40*) and enveloped virus such as *Sindbis virus, HSV type I and VSV* have been concentrated with magnetic beads (up to 100 times for DNA viruses and up to 1000 fold for RNA viruses) and the sensitivity of virus detection by PCR was enhanced *(Satoh et al. 2003. J. Virol. Methods 114: 11-19)*. However, the authors reported a reduced infectivity of the viruses associated with magnetic beads having a size of 0.8µm in diameter and coupled to a

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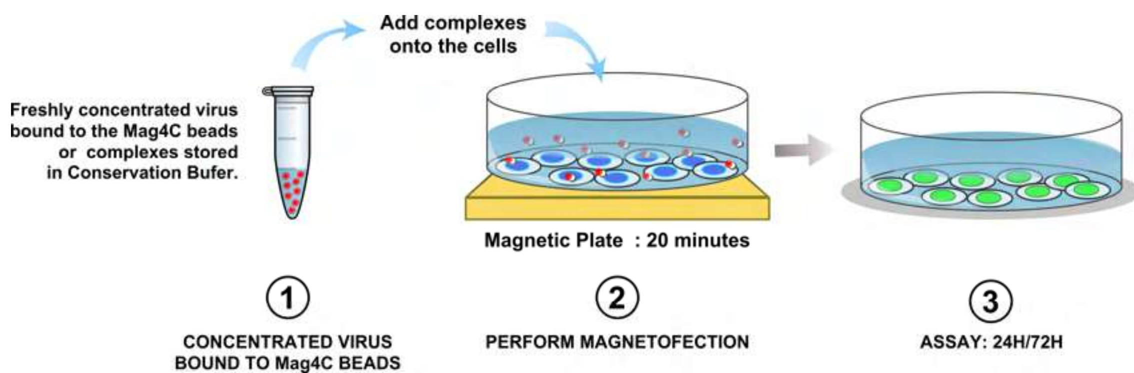
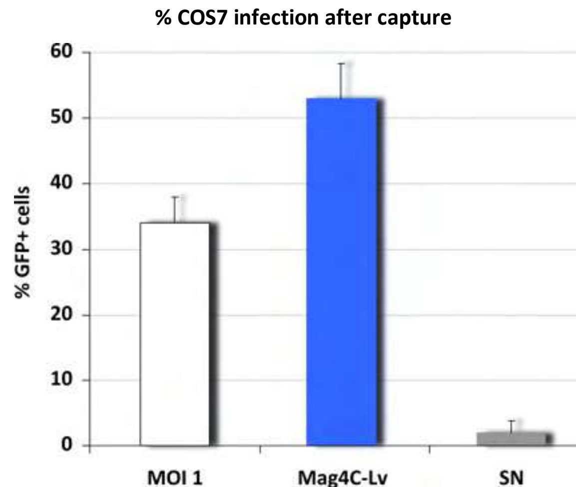
very large polymer. This is not surprising since the beads size precludes internalization and infection, while the polymer biological activity is extremely low. In contrast, Mag4C-Lv beads small nanoparticles formulation concentrates viruses in the same way and improves viruses' infectivity as demonstrated for various types of viruses due to their small size and the particularly active coated polyelectrolyte.

- For cell culture or in vivo infection. Magnetic nanoparticles improve *in vitro* and *in vivo* lentivirus infectivity. Once captured, concentrated and re-suspended, lentiviruses bound to Mag4C-Lv beads can be used directly to infect cells using the Magnetofection™ technology. Magnetofection™ uses magnetic force to drive the virus associated with magnetic particles towards the target cells. In this way, the complete applied dose (100%) of virus gets concentrated onto the cells surface very rapidly. This method allows concentrating the entire viral dose on the cells quickly, accelerating the transduction process and infecting non-permissive cells. The virus infection efficiency is considerably increased and virus adsorption can be synchronized without modification of the virus. Targeted/confined transduction to specific area (magnetic targeting) can also be accomplished.



HIV-SFFV-GFP lentivirus was captured and concentrated using Mag4C-Lv Kit as described above. Magnetized lentivirus (Mag4C-Lv) and supernatant (SN) were tested for infectivity on COS7 cells. Transduction efficiency was compared to virus alone (MOI 1). The GFP + cells (%) were analyzed 24 hours post-transduction by FACS.



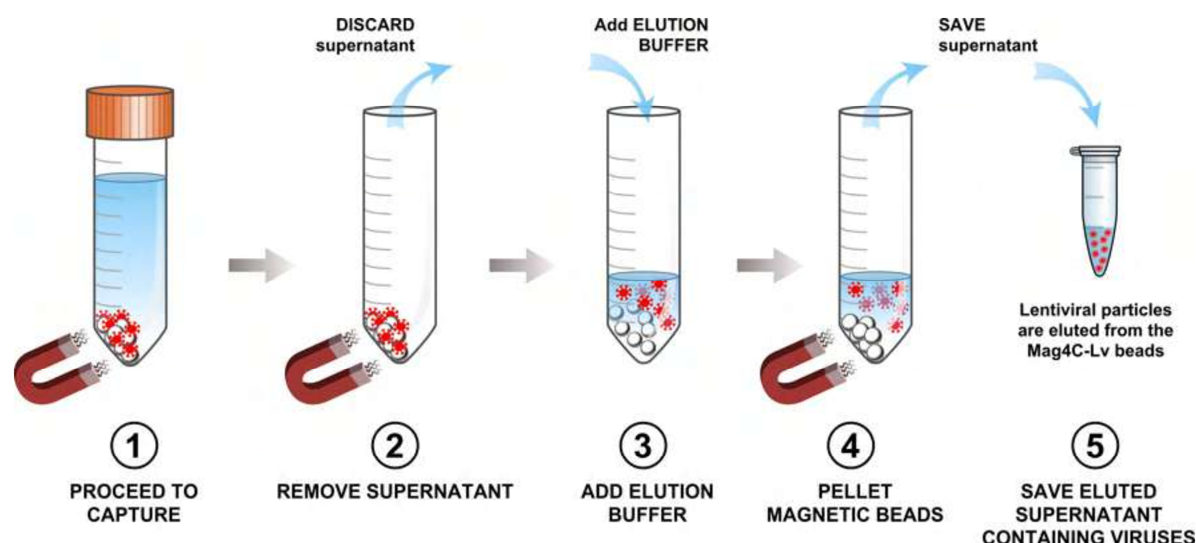
Results show that Magnetofection procedure can be followed after capture and concentration using Mag4C-Lv Kit and confirm that transduction efficiency is largely increased in comparison to virus alone.

In summary, keeping the Mag4C-Lv beads associated to the viruses after concentration:

1. Increase infection efficiency
2. Allow adjusting the medium or buffer (remove or reduce serum, lower salt etc.) easily
3. Improve sensitivity of molecular biology assays
4. Do not alter virus viability

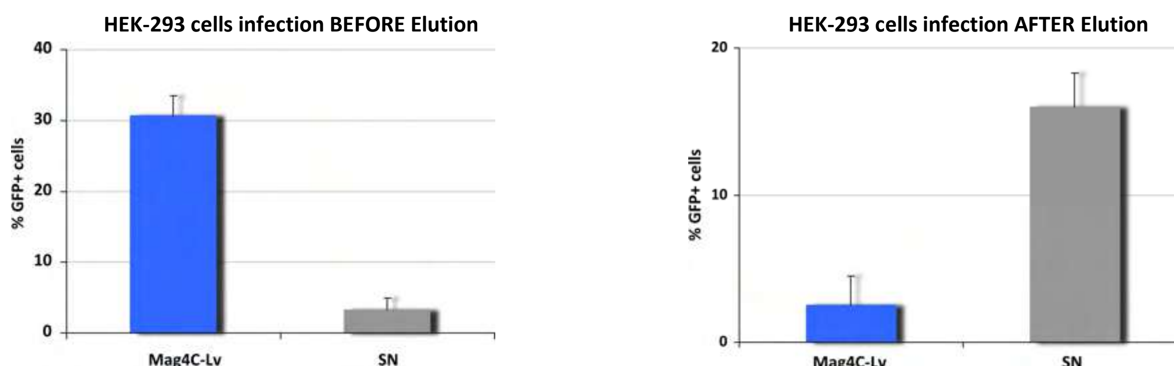
3- Elution of virus after capture and concentration (removal of Mag4C-Lv beads)

This step is optional. You can choose to keep the beads associated with the virus or remove them and have a "beads-free" concentrated virus. The **Elution Buffer** (1) is highly effective in separating viruses from Mag4C-Lv beads, and (2) allows concentration of eluted viruses.



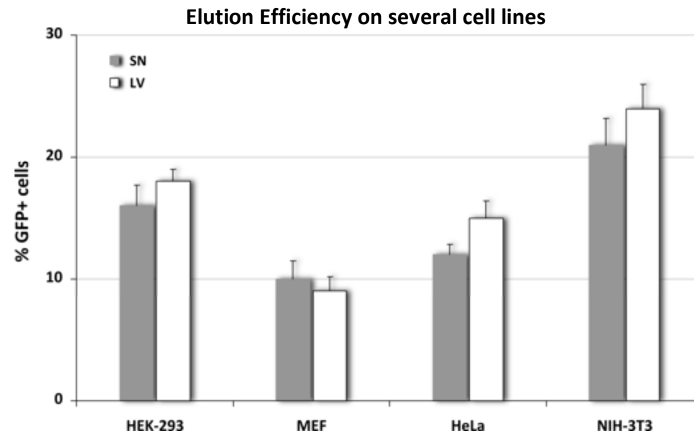
Elution efficiency

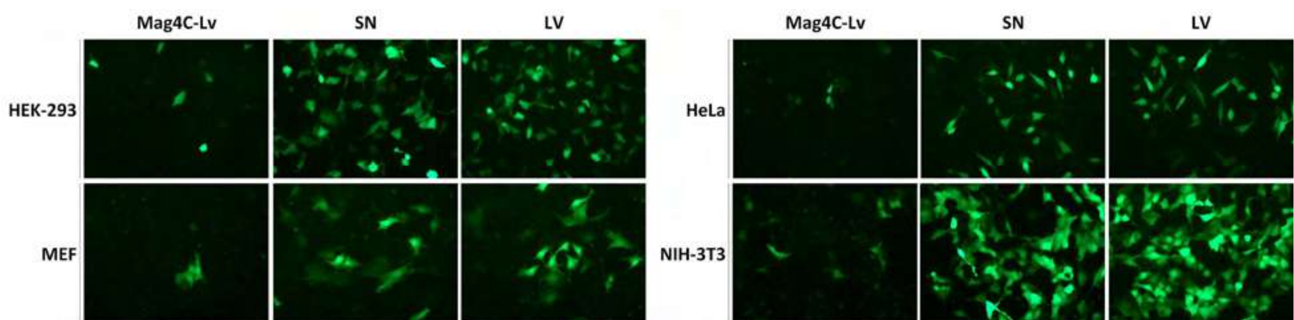
The capture was performed as previously described. Lentiviruses/Mag4C-Lv beads complexes were then suspended in the **Elution Buffer** to separate lentiviruses from beads. After 5 min of incubation at RT, Mag4C-Lv beads were retained on the Magnetic Separation Rack while the eluted supernatant was saved. Then, the Mag4C-Lv beads were re-suspended in PBS and both elution buffer (containing the free virus) and Mag4C-Lv beads were tested for transduction efficiency. % of GFP positive HEK-293 cells was determined after 24h by FACS.



Results demonstrate that Mag4C-Lv beads were no more infectious after the elution procedure. The lentiviruses have thus been captured, concentrated and eluted efficiently.

Similarly, HEK-293, MEF, HeLa and NIH-3T3 cells were transduced at a MOI of 1 with either 1) eluted lentiviral particles (SN), or 2) an untreated virus (LV) was used as control. % of GFP positive cells was determined after 24 hours by flow cytometry.



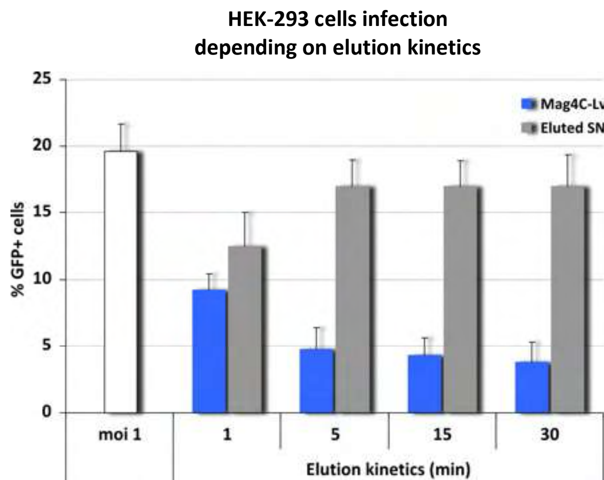


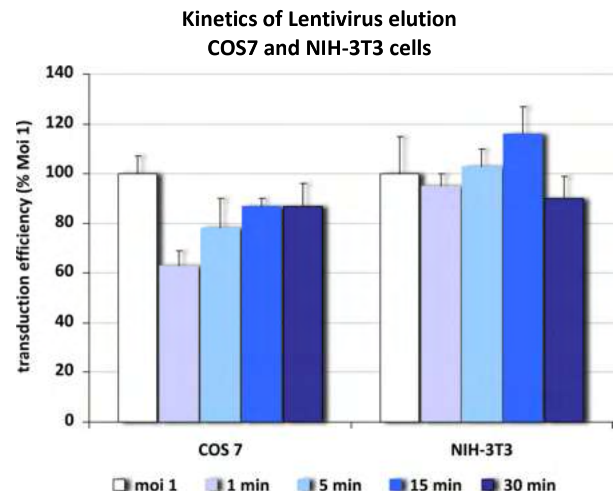
10^6 viral particles were mixed with 20 µL of Mag4C-Lv beads and complexed during 15 min at RT. After capture, lentiviruses were incubated 5 min in 100 µL of the **Elution Buffer** at 5 different concentrations (0.1X to 1X) or PBS. The supernatants were then used to infect HEK-293 cells and an untreated virus was used as control (MOI 1). The % of GFP positive cells was determined after 24h hours by flow cytometry.

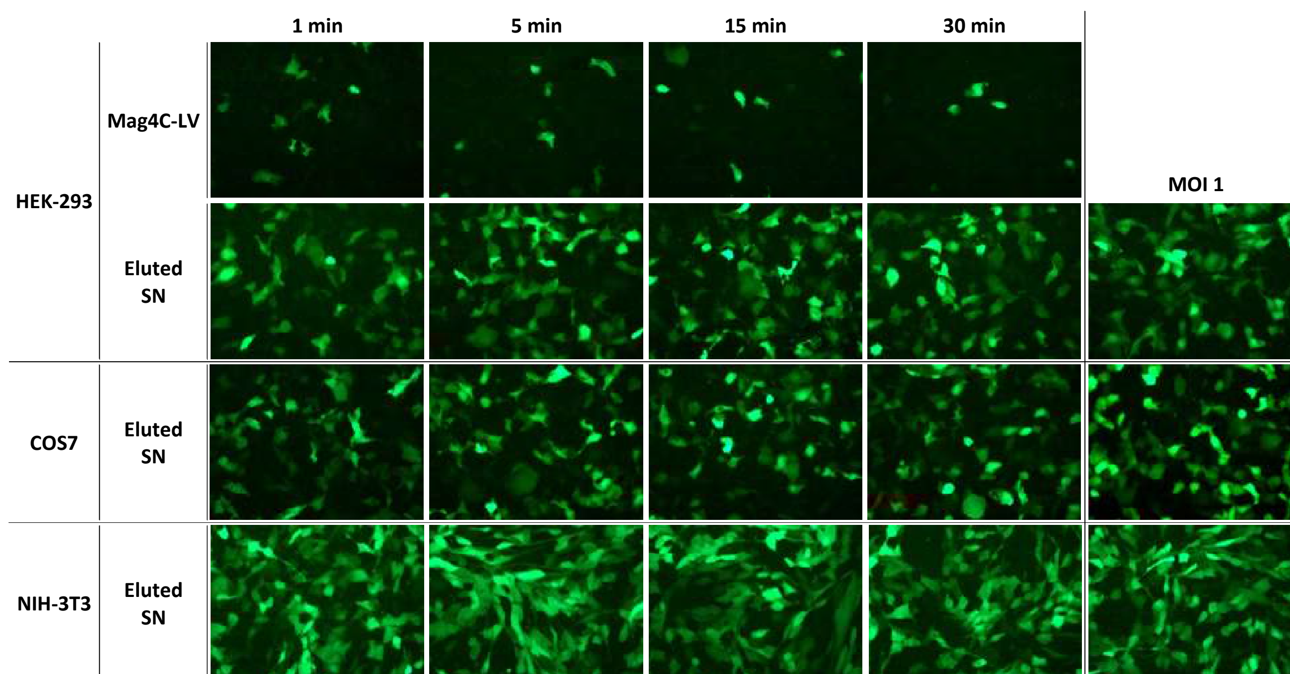
Results show that viruses keep their infectivity after the capture / concentration / elution procedures. All viruses were effectively eluted from Mag4C-Lv beads that were then non infectious. Moreover, about 80-99% of the initial virus dose was recovered after capture, concentration and elution.

Rapid kinetics of elution

Elution buffer allows eluting captured lentiviruses within minutes. After capture, lentiviruses were eluted from Mag4C-Lv beads in 100 µL Elution Buffer during 1 to 30 min. Eluted supernatants were used for infecting HEK-293 cells. % of GFP positive cells was determined after 24h by FACS and fluorescence microscopy.





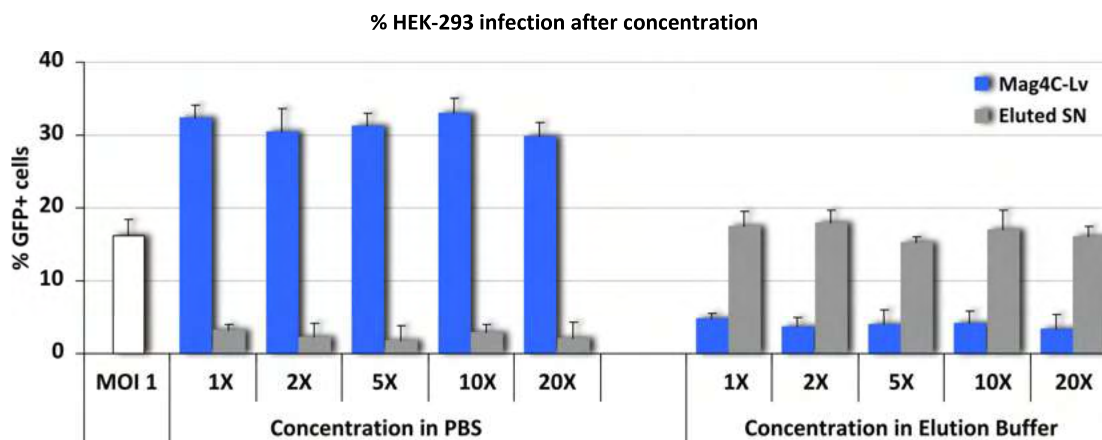


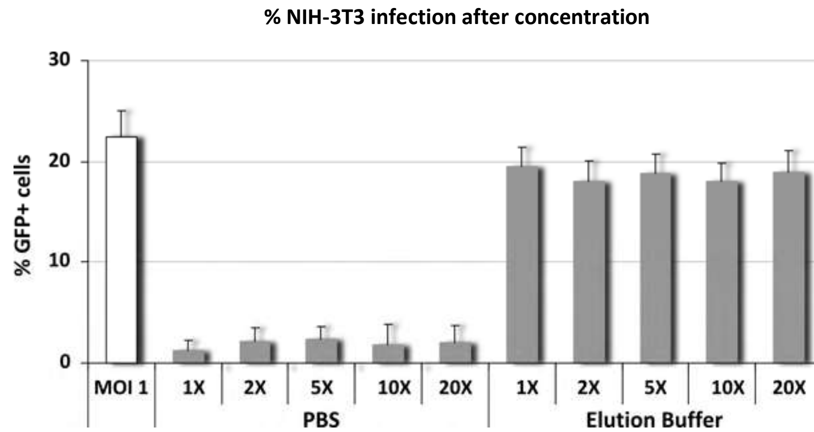
Results show that Elution of captured lentivirus occurs within minutes. The elution procedure reaches its maximum of efficiency after 5 min of incubation.

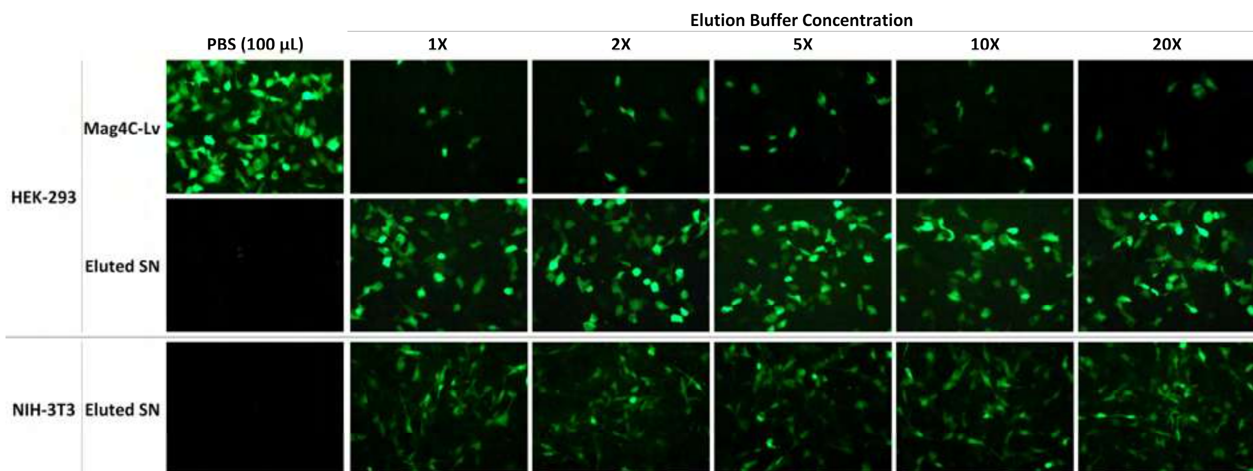
Concentrate the lentiviral particles with Elution Buffer

To easily concentrate the captured viruses during elution process, simply use low elution volumes for concentration. In this way, lentiviral particles will be released from the Mag4C-Lv beads in smaller volumes resulting in the concentration of the lentivirus.

10^6 viral particles suspended in 100 µL DMEM were mixed with 20 µL of Mag4C-Lv beads and complexed for 15 min at RT. After capture, lentiviruses were eluted from Mag4C-Lv beads by 5 min incubation in 100µL, 50µL, 20µL, 10µL or 5µL of **Elution Buffer** (i.e. 1X, 2x, 5x, 10X and 20x concentration respectively). Eluted supernatants and an untreated virus (control; MOI 1) were used to infect HEK-293 and NIH-3T3 cells. The % of GFP positive cells was monitored after 48h by flow cytometry and fluorescence microscopy. As a control for elution, same procedure was applied using PBS.







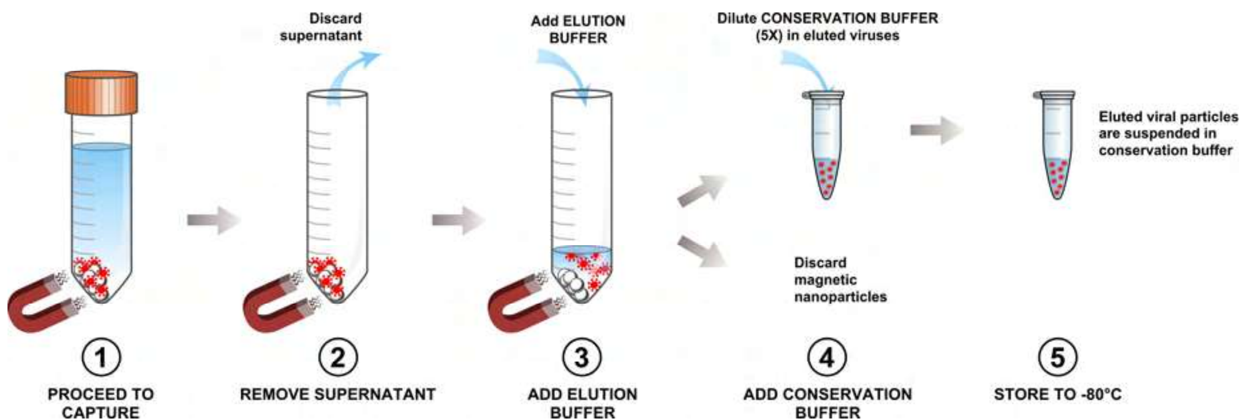
Results demonstrate that captured, eluted and concentrated lentivirus is as infectious as untreated virus.

In summary, the Elution of virus from the Mag4C-Lv beads is:

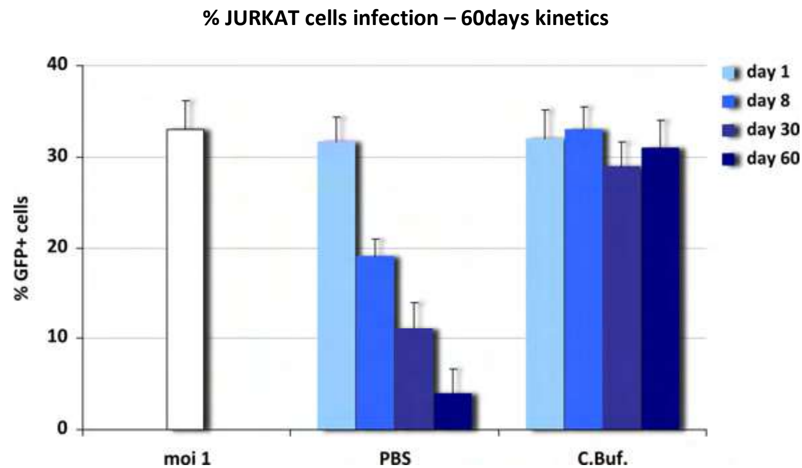
1. Straightforward, easy and very efficient
2. Powerful, 80-99% of the initial virus dose is captured, eluted and concentrated
3. Versatile, elution buffer can be used as concentration buffer

4- Conservation/storage of virus

Conservation Buffer does not hamper infection efficiency of lentivirus

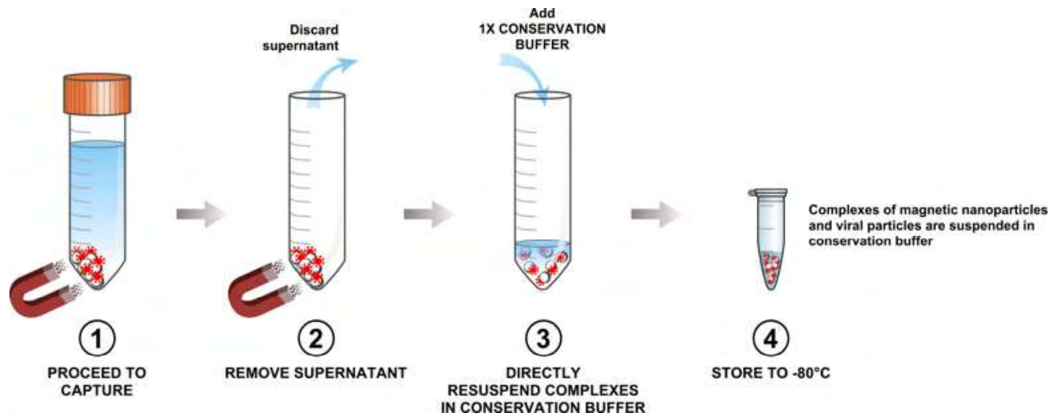


After the elution procedure as described above, **Conservation Buffer** or PBS was added (1x final) to the eluted lentiviral solution and stored at -80°C for several days. At day 1, 8, 30 and 60 after storage, viruses in PBS or Conservation Buffer-(C.Buf.) were used to infect Jurkat cells at a MOI of 1. A fresh viral suspension (MOI 1) was used as control. The % of GFP positive cells was determined 48h post-transduction by flow cytometry.

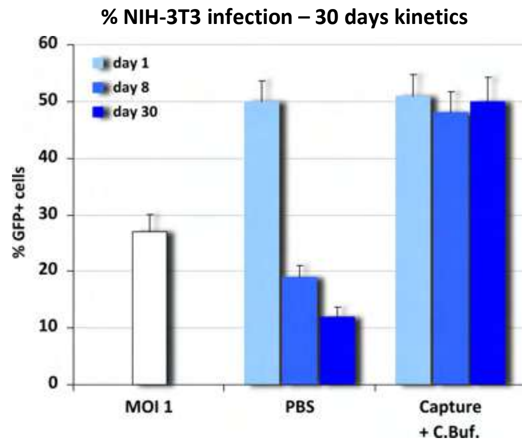


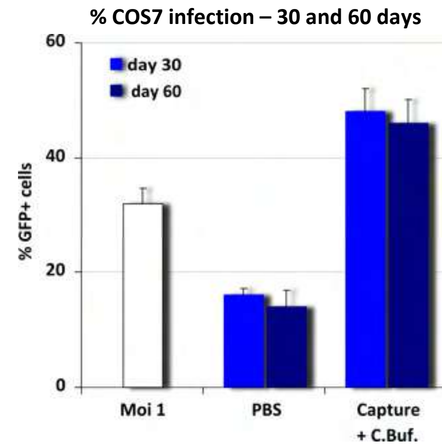
Results show that lentivirus infectivity remains intact after storage in Conservation Buffer whereas lentivirus stored in PBS readily loses infectivity.

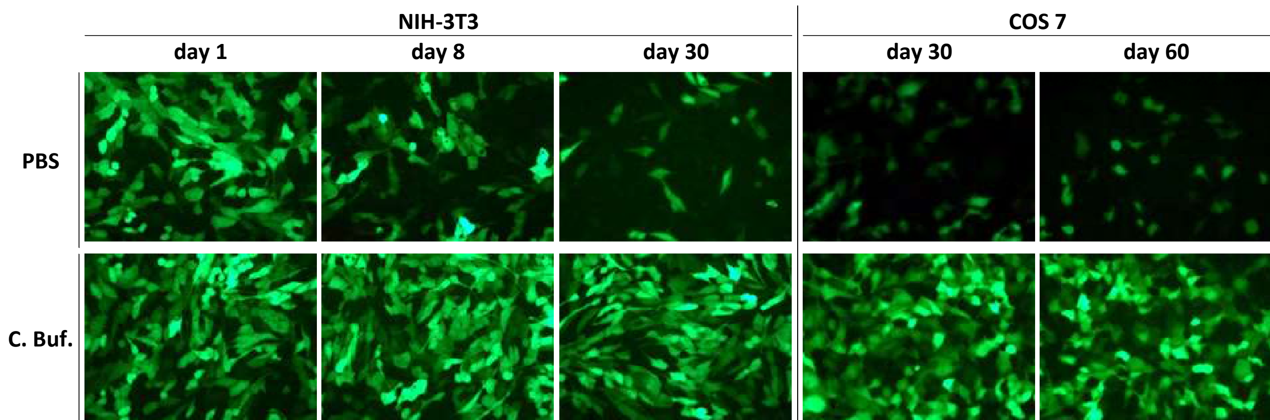
<u>Conservation Buffer is suitable for lentivirus/Mag4C-Lv beads complexes</u>



Eluting the virus from the Mag4C-Lv beads is optional. Virus/Mag4C-Lv complexes can be directly suspended in Conservation Buffer for storage. After capture, virus/Mag4C-Lv complexes were stored at -80°C in PBS or Conservation Buffer (C.Buf.) for various periods of time. After 1, 8 and 30 days of storage, COS7 and NIH-3T3 cells were infected at a MOI of 1 with virus/Mag4C-Lv complexes stored in PBS (PBS) or in conservation buffer (Capture + C.Buf.). As control, untreated virus (freshly produced at MOI1) was used. The % of GFP positive cells was determined after 48h by flow cytometry.







Results show that Conservation Buffer can be directly added to the Mag4C-Lv beads-bound viruses without hampering neither the beads properties nor the lentivirus infectivity.

In summary, the Conservation Buffer:

1. Preserves lentivirus infectivity during storage
2. Minimizes the loss of viral titers during storage and freeze/thaw cycles
3. can be used with Mag4C-Lv beads

Bibliographic references

Two recent papers described the use of OZ Biosciences magnetic nanoparticles for virus capture:

- Wang YJ et al., 2010, J Virol Methods. 2010 May; 165(2):230-7
- Lu M et al., Antimicrob Agents Chemother. 2012 Jun; 56(6):3324-35.

The authors highlighted the following features:

1. isolation of virus from high concentration of infection inhibitors in the supernatant
2. shortening of the entire process from virus isolation to drug susceptibility
3. higher tolerance of captured virus to freeze/thaw cycle without loosing any infectivity.