Viral Applications

Mag4C-Lv

To capture, concentrate and store Lentiviruses and Retroviruses

Protocol

Magnetofection Technology
This reagent needs to be used with a magnetic plate

OZBIOSCIENCES
The art of delivery systems

IMPORTANT NOTES – Before you begin

- ✓ ***Mag4C-Lv Kit*** is 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.
- ✓ **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.

CAUTION: **The kit do not contain the Magnetic Separation Rack that must be purchased separately**

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 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 volumes
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 cycle
3. Compatible with magnetic nanoparticles

For additional information and protocols (optimization, scaling, co-transfection…) tips, troubleshooting or other applications

www.ozbiosciences.com

Any questions?

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Package content	LTK11200 Mag4C-Lv Trial kit ✓ Mag4C-Lv beads (0.2 mL) ✓ Elution Buffer (5 mL) ✓ Conservation Buffer (0.2 mL) Number of assays*: Up to 20 LKC11000 Mag4C-Lv kit ✓ Mag4C-Lv beads (1mL) ✓ Elution Buffer (5mL) ✓ Conservation Buffer (1mL) Number of assays*: Up to 100 LKC11010 Mag4C-Lv kit ✓ Mag4C-Lv beads (10mL) ✓ Elution Buffer (50mL) ✓ Conservation Buffer (10mL) Number of assays*: Up to 1000 LKC11050 Mag4C-Lv kit ✓ Mag4C-Lv beads (50mL) ✓ Elution Buffer (250mL) ✓ Conservation Buffer (50mL) Number of assays*: Up to 5000 *Number of captures based on 1 mL of virus preparation
Shipping conditions	The kit is shipped at Room Temperature.
Storage conditions	Mag4C-Lv beads, Elution Buffer Storage and Conservation Buffer Storage: +4°C – *Do not freeze the Mag4C-Lv beads!* – *Do not add anything to the Mag4C-Lv beads and buffers!* Kit components are stable for at least one year at the recommended storage temperature.
Shelf life	1 year from the date of purchase when properly stored and handled
Important notice	For research use only. Not for use in diagnostic procedures.

Applications

1. Virus Types

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

2. Downstream Biological Assays

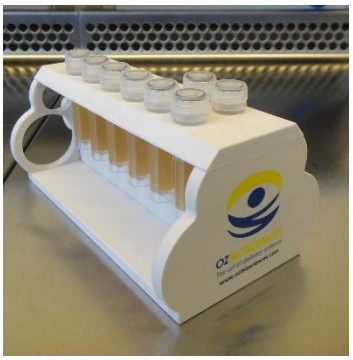
After magnetic capture and concentration, viruses can be used for multiple assays. For instance, **they can be used for PCR, western blot, ELISA, *in vitro* and *in vivo* infection, etc.**

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 (free of beads), and virus in conservation buffer have all 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.

3. Magnetic separation rack and Magnetofection™ apparatus

Mag4C-Lv Kit requires appropriate magnetic fields for concentrating magnetized viruses.



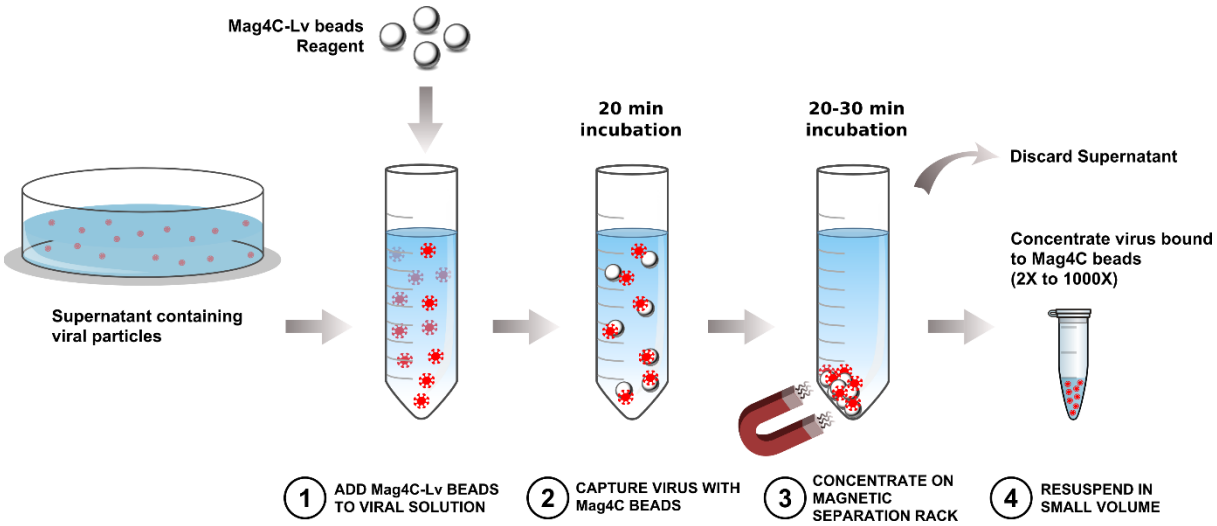
The Magnetic Separation Rack (#MSR1000, photo), is designed for 50, 15 or 1.5 mL tubes. It can hold 12 standard microtubes, two 15 mL and two 50 mL tubes. The Magnetic Separation Rack is required for capture, concentration, washing and elution.

If Mag4C-Lv beads are kept bound to viruses and to take advantage of the Magnetofection™ technology for the downstream cell culture or *in vivo* assays, a magnetic plate or magnets are needed. OZ Biosciences provide specific magnetic plates for Magnetofection™: 96-magnets plate (#MF10096), super magnetic plate (#MF10000) and mega magnetic plate (#MF14000) and specific magnets set for *in vivo* applications (#IV-MAG1).

4. General Considerations

The instructions given below represent typical protocols that were applied successfully to **Capture**, **Concentrate**, **Elute**, and **Conserve** freshly produced or purchased viruses. Our R&D team has tested and optimized the **Mag4C-Lv Kit** in order to provide you with the most straightforward and efficient procedure. Therefore, we suggest you to start by following our general protocol as guidelines to obtain good data rapidly. Thereafter, we recommend optimizing the conditions to achieve the best performance. Indeed, optimal conditions vary from one virus production to another and are highly dependent upon the type of virus used, its titer, the composition of the viral solution, and cell culture conditions. We advise you to optimize the experimental condition parameters as described in the Appendix in order to achieve the best effects.

5. Capture/Concentration Protocol



The protocol is simple: 20 µL of **Mag4C-Lv** beads are sufficient to bind 1x10^6 infectious viruses with almost 80-99% efficiency. Please refer to the **Table 1** for the suggested **Mag4C-Lv** beads volume according to the virus titer.

Depending on the virus type, the total virus quantity (particles and infectious), and the complexity of the medium, this protocol would have to be adjusted (see appendix). It is recommended to raise the volume of **Mag4C-Lv** beads for complex medium (complete culture medium, organic fluids…).

Table 1 Recommended volume of **Mag4C-Lv** beads according to the number of infectious viruses

Viral Preparation (mL)	Mag4C-Lv beads (µL) "Starting Point" *	Mag4C-Lv beads (µL) Suggested range of testing
≤ 2 mL	20 µL	10 µL – 40 µL
> 2mL	10 µL / mL	5 µL – 20 µL / mL

* for high titer viral solution (≥ 10^7 infectious viruses / mL), we recommend using 1.5 or 2 times the suggested volume.

Important: The suggested volume of Mag4C-Lv beads for capture is related to infectious particles and not physical viral particles.

1) Add 20 µL of **Mag4C-Lv** beads into the virus preparation ≤ 2 mL or 10 µL / mL of **Mag4C-Lv** beads for virus preparation > 2 mL. It may be necessary to adjust the volume of Mag4C-Lv depending on the composition of the virus solution (see Table 1).
2) Incubate 20-30 min at room temperature to capture viruses.
3) Place the tube 15 to 30 min onto the Magnetic Separation Rack to concentrate the virus/Mag4C-Lv beads complexes. Incubation time will depend on the tube volume (see Table 2). Then, discard supernatant.

NOTE: Brown pellet should be visible on the side of the tube near the magnets.

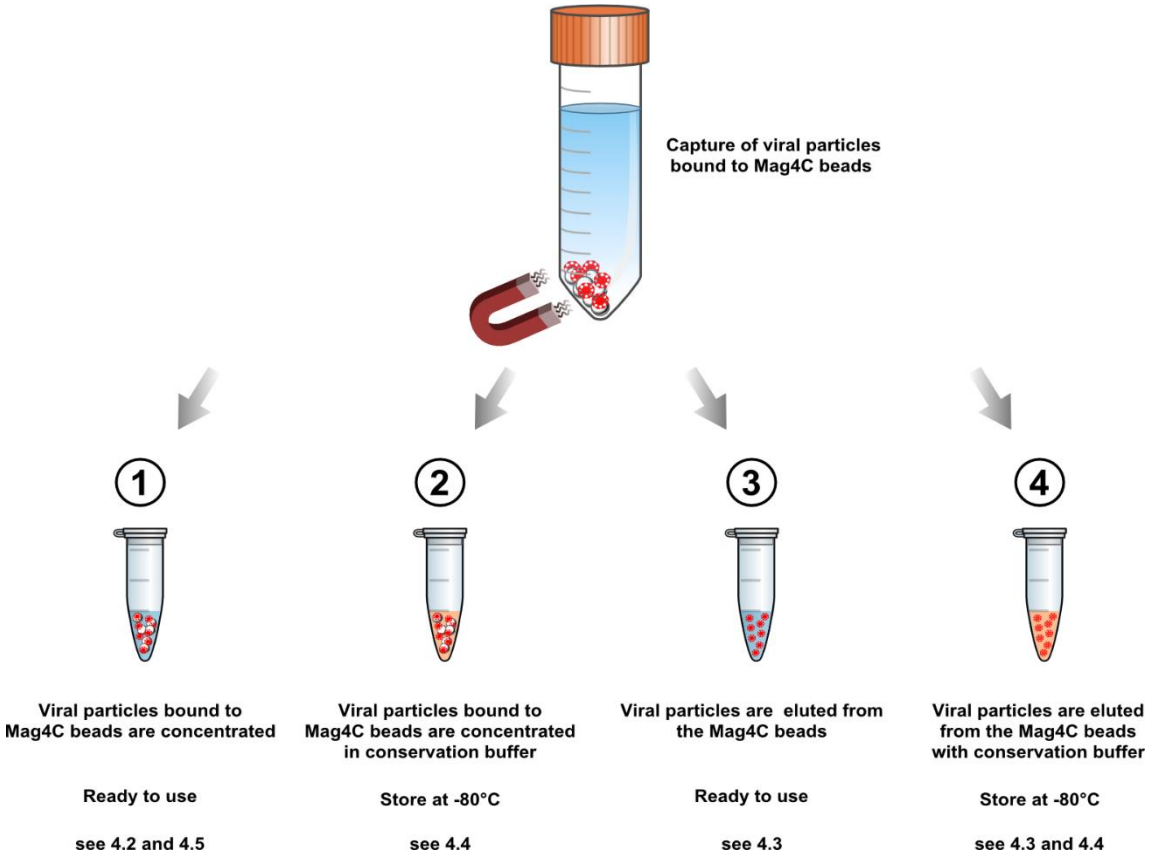
OPTIONAL: A washing procedure can be performed after this step:

i. Keep the tube on the Magnetic Separation Rack and slowly add PBS (same volume as the initial medium).
ii. Incubate 5 min on the Magnetic Separation Rack.
iii. Discard the supernatant.
iv. Proceed to step 4

Table 2 Recommended incubation time on the Magnetic Separation Rack according to the volume

Tube volume	Time on Magnetic Separation Rack
1 mL	15 min
10 mL	20 min
50 mL	30 min

4) The virus/Mag4C-Lv beads complexes can be used according to the following 4 options



(1) viruses are concentrated with Mag4C-Lv beads into smaller volumes of PBS (with Ca^{2+} and Mg^{2+}) or complete cell culture medium and used immediately for assay (for example see 4.5). Determine the appropriate volume of PBS/medium to add according to the expected final concentration.

(2) viruses are concentrated with Mag4C-Lv beads into smaller volumes of Conservation Buffer for long term storage (see 4.4)

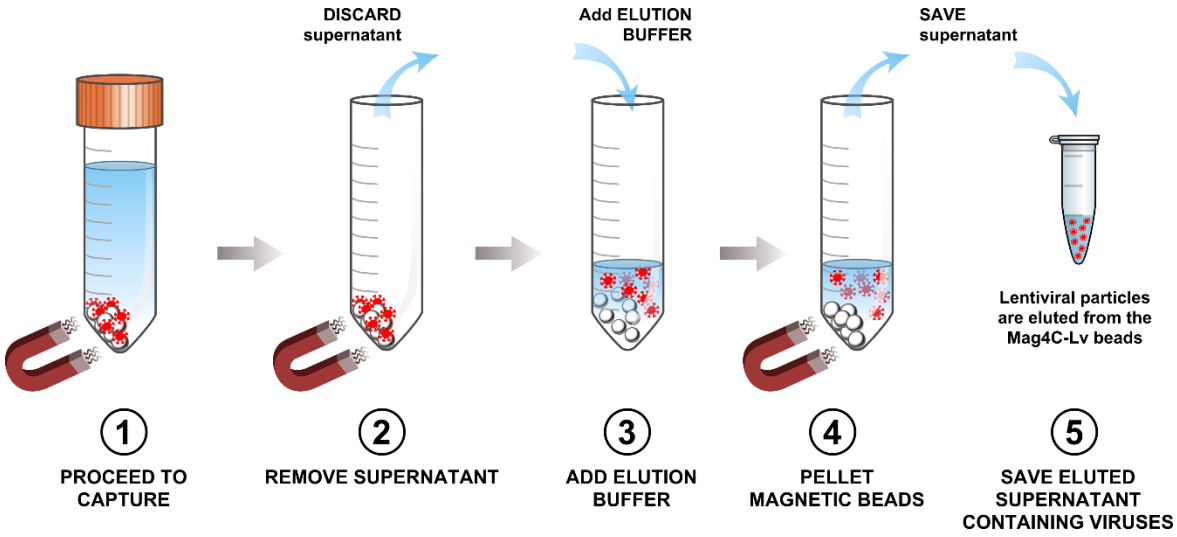
(3) viruses are eluted from Mag4C-Lv beads (see 4.3), concentrated into smaller volumes of Elution Buffer and used immediately for assay

(4) viruses are eluted from Mag4C-Lv beads (see 4.3), concentrated into smaller volumes of Elution Buffer plus Conservation Buffer for long term storage (see 4.4)

Keeping the Mag4C-Lv beads bound to viruses offers several advantages especially in terms on *in vitro* and *in vivo* infectivity as it allows using the Magnetofection™ technology (see 4.5):

4.3. Elution Procedure

This step is optional. You can choose to keep the nanobeads associated to the virus or remove them and have a "beads-free" concentrated virus. The **Elution Buffer** is a ready-to-use buffer, specifically designed to elute lentiviruses bound to the Mag4C-Lv nanobeads without impairing their infectious properties.



Important Note: For storage of **eluted** viruses, go directly to the section 4.4

1) Proceed to capture the virus as previously described and discard the supernatant (see 4.2 – steps 1 to 3).

Add the Elution Buffer to the virus/Mag4C-Lv beads complexes. Determine the appropriate volume of Elution Buffer to add according to the expected final concentration (see Table 3). For example, if the initial virus solution is 1mL and you want to concentrate 10 fold, then add 100µL of Elution Buffer. use volumes of elution buffer recommended to concentrate 10X and adjust to initial volume with PBS or culture medium so as not to concentrate viral particles.

Table 3 Volume of Elution Buffer for concentration and immediate use

Starting viral solution	Expected concentration 100X	Expected concentration 50X	Expected concentration 10X	Washing or medium exchange
1 mL	10 µL	20 µL	100 µL	1 mL
5 mL	50 µL	100 µL	500 µL	5 mL
10 mL	100 µL	200 µL	1 mL	10 mL
50 mL	500 µL	1 mL	5 mL	50 mL

2) After addition of the Elution Buffer, incubate for 5 to 10 min at RT.
3) Place the tube on the Magnetic Separation Rack and incubate 10 to 30 min at RT.
NOTE: Adjust incubation time on the Magnetic Separation Rack according to the volume (refer to table 2).

4) Save the supernatant containing viruses and discard pellet of Mag4C-Lv nanobeads.
5) The concentrated viruses solution can be used for downstream assay or proceed to section 4.4 for storage.

6. Conservation Procedure

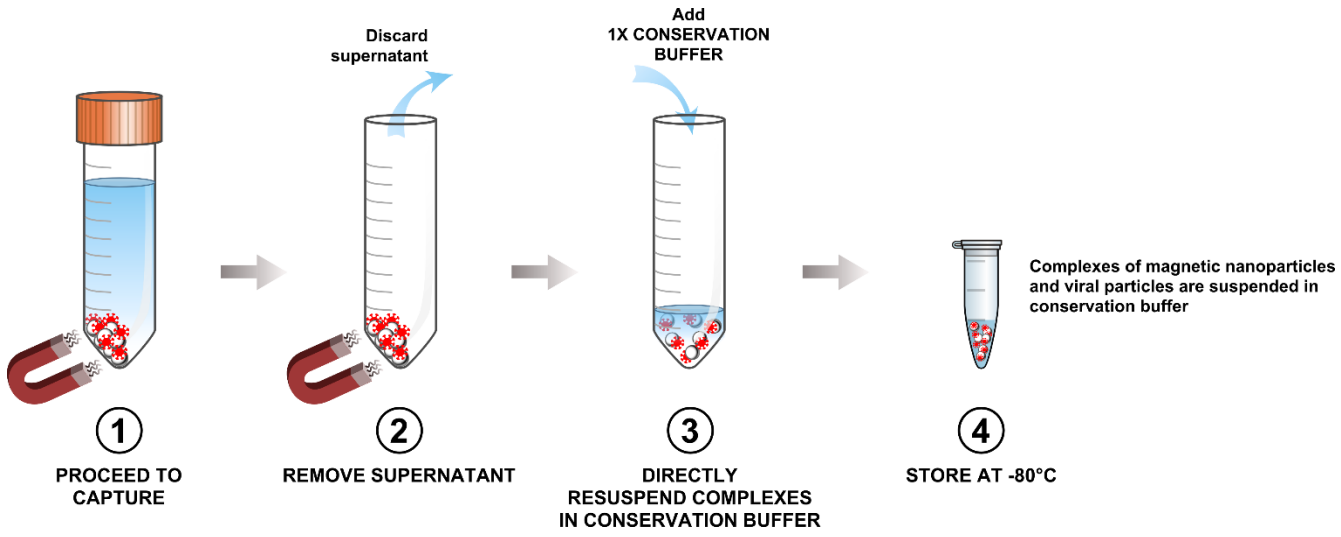
Conservation procedure with Mag4c-Lv nanobeads.

Conservation buffer is 100% compatible with Mag4C-Lv nanobeads. In this way, Conservation Buffer can be added right after the capture procedure. Conservation Buffer allows virus storage for several months at -80°C and preservation of the virus titer upon freeze/thaw cycles.

Conservation Buffer preparation: Dilute 1 volume of the Conservation Buffer (5x) in 4 volumes of PBS (1X final). For 100 µL conservation buffer, add 20 µL of buffer to 80 µL of PBS.

1) Proceed to capture the virus as previously described and discard the supernatants (see 4.2 – steps 1 to 3)
2) Remove the tube form the Magnetic Separation Device
3) Add freshly prepared conservation buffer to the complexes. To concentrate the virus solution, use smaller volume of buffer.
4) Store the complexes at -80°C.

NOTE: To reduce freezing/thawing cycles, it is recommended to aliquot virus for long term storage.



Conservation procedure after elution.

Conservation Buffer can be added right after the elution step. Conservation Buffer allows virus storage for several months at -80°C and preservation of the virus titer upon freeze/thaw cycles.

1) Proceed to capture the virus as previously described and discard the supernatants (see 4.2 – steps 1 to 3)
2) Add the Elution Buffer to the virus/Mag4C-Lv beads complexes (see Table 4 for Elution Buffer volume).

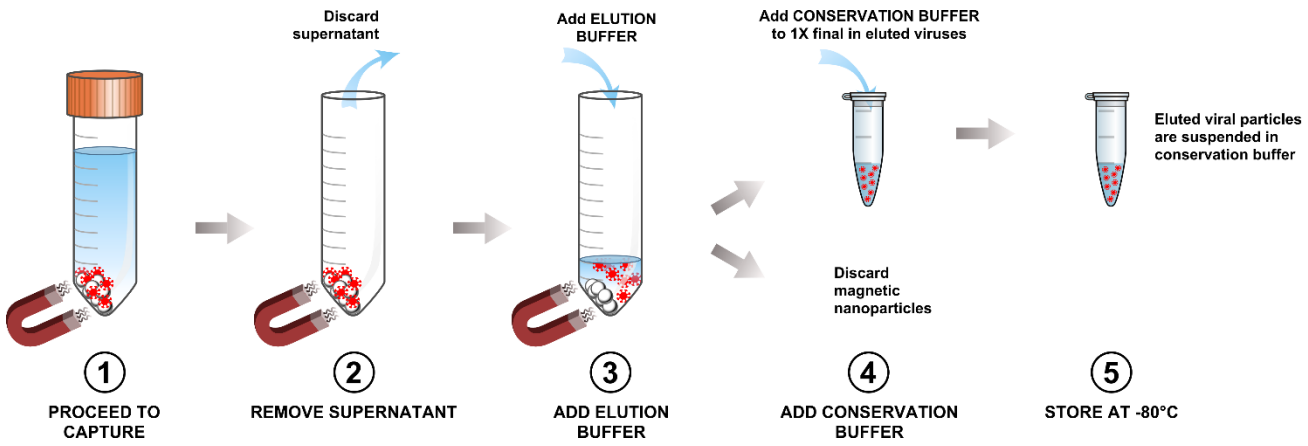
Table 4 Volume of Elution Buffer (EB) and Conservation Buffer (CB) for concentration and storage

Starting viral solution	Expected concentration 100X		Expected concentration 50X		Expected concentration 10X		Washing or medium exchange	
	EB	CB	EB	CB	EB	CB	EB	CB
1 mL	8 µL	2 µL	16 µL	4 µL	80 µL	20 µL	800 µL	200 µL
5 mL	40 µL	10 µL	80 µL	20 µL	400 µL	100 µL	4 mL	1 mL
10 mL	80 µL	20 µL	160 µL	40 µL	800 µL	200 µL	8 mL	2 mL
50 mL	400 µL	100 µL	800 µL	200 µL	4 mL	1 mL	40 mL	10 mL

3) After addition of the Elution Buffer, incubate for 5 to 10 min at RT.
4) Place the tube on the Magnetic Separation Rack and incubate 10 to 30 min at RT.
NOTE: Adjust incubation time on the Magnetic Separation Rack according to the volume (refer to table 2).

5) Save the supernatant containing viruses and discard pellet of Mag4C-Lv nanobeads.
6) Add the Conservation Buffer (5x) directly to the eluted viruses solution for a 1X final concentration. See Table 4.
7) Store virus at -80°C

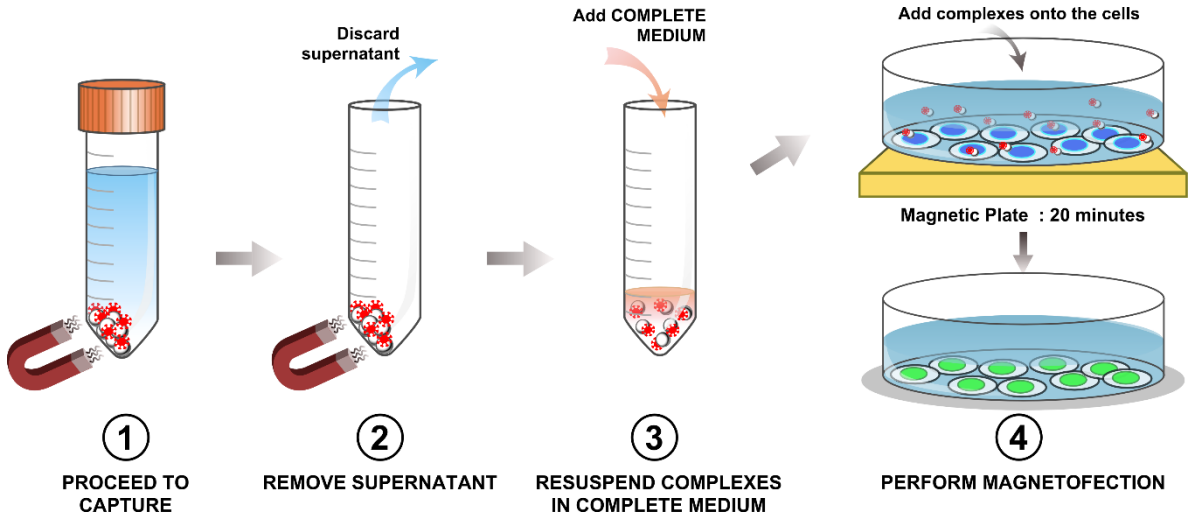
NOTE: To reduce freezing/thawing cycles, it is recommended to aliquot virus for long term storage.



7. Magnetofection™ Procedure

Keeping the Mag4C-Lv beads bound to viruses offers the Magnetofection™ technology advantages. 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.

After capture, concentration and storage (with the nanobeads), the Magnetofection procedure can be performed for *in vitro* transduction or *in vivo* infection. Specific protocols are available directly on our website for *in vitro* and *in vivo* experiments.



Magnetofection *in vitro* on adherent cells

1) Perform the capture and concentration of the virus as described above (see 4.2, steps 1-4) or thaw the virus/Mag4C-Lv beads complexes vial (4.4 conservation with the beads).
2) This protocol is given for a 24-well plate format; refer to the Magnetofection protocol for other sizes of culture dishes.
3) Plate the cells the day prior transduction. Best results are achieved if cells are at least 60-80 % confluent at the time of Magnetofection (if required refer to the suggested cell number in the Magnetofection protocol).
4) Add the virus/Mag4C-Lv beads complexes to the cells at the desired MOI in a drop wise manner.
5) Mix by gently rocking the plate to ensure correct dispersion of magnetic complexes within culture medium.
6) Place the cells upon the specific magnetic plate (see section 3) for 20-30 minutes.
NOTE: Optionally after this incubation, a medium change can be performed while maintaining the magnetic plate under the cell culture.

7) Remove the magnetic plate and cultivate the cells under standard conditions until evaluation of the transduction experiment.
NOTE: Optionally a medium change can be performed after 24 hours.

- For suspension cells, please refer to the Magnetofection protocol (ViroMag and ViroMag R/L or Viro-MICST)
- For *in vivo*, please refer to the *in vivo* Magnetofection protocol

NOTES

Additional products

- **Mag4C-Lv Kit** for for capturing, concentrating and storing.

- **Viro-PEG Lentivirus Concentrator** for the capture and concentration of lentiviral particles, providing an easy and straightforward method to efficiently concentrate lentiviral particles without using ultracentrifugation.

Purchaser Notification

Limited License

The purchase of the Mag4C-Lv Kit grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in this protocol). This reagent is intended for in-house research only by the buyer. Such use is limited to the transfection of nucleic acids 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. Separate licenses are available from OZ Biosciences for the express purpose of non-research use or applications of the Mag4C-Lv Kit. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact us at OZ Biosciences. Buyers may end this License at any time by returning all Mag4C-Lv Kit reagents and documentation to OZ Biosciences, or by destroying all D-Luciferin components. Purchasers are advised to contact OZ Biosciences with the notification that a Mag4C-Lv Kit is being returned in order to be reimbursed and/or to definitely terminate a license for internal research use only granted through the purchase of the kit(s). This document covers entirely the terms of the Mag4C-Lv Kit research only license, and does not grant any other express or implied license. The laws of the French Government shall govern the interpretation and enforcement of the terms of this License.

Product Use Limitations

Mag4C-Lv Kit 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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