Viral Applications

ViroMag RL™

Transduction enhancer reagent

For Lentivirus and retrovirus

Protocol

Magnetofection Technology

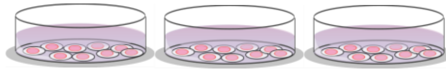
This reagent needs to be used with a magnetic plate

OZBIOSCIENCES
The art of delivery systems

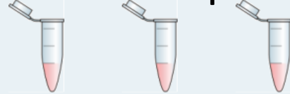
ViroMag R/L Quick Protocol

To find the ideal conditions, **ViroMag R/L** must be tested at several amounts according to the type of culture dish/plate independently to the MOI used. Adapt your MOI depending of your viral vector and the type of cells used.

1. Seed cells to be at 70% confluent the day of transfection*



2. Prepare 4 identical tubes of viral particles (example for MOI of 1)

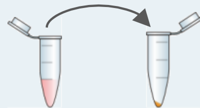


96 well plate	24 well plate	6 well plate
For $1x10^4$ cells per well, dilute $1x10^4$ infectious units in 50µL serum-free medium or buffer* x4	For $1x10^5$ cells per well, dilute $1x10^5$ infectious units in serum-free medium or buffer* 100µL x4	For $5x10^5$ cells per well, dilute $5x10^5$ infectious units in serum-free medium or buffer* 200µL x4

3. Prepare 4 tubes of ViroMag R/L (with different amounts of magnetic beads)*
The volumes of ViroMag R/L to be tested is fixed and is the same for any MOI

96 well plate	24 well plate	6 well plate
0.5µL/1µL/1.5µL/2µL in an empty microtube	1.5µL/3µL/6µL/9µL in an empty microtube	7.5µL/15µL/30µL/45µL in an empty microtube

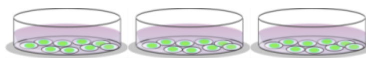
4. Mix each tube of viral particles (step 2) to each tube of ViroMag R/L (step 3)*



5. Incubate 5 to 20 min at room temperature or on ice

6. Distribute each mix drop by drop onto the cells to insure uniform distribution & then incubate the cells 20 min. on the magnetic plate

7. Remove the cells from the magnetic plate and incubate cells for 24 to 72h at 37°C until evaluation of transgene expression*



8. Choose the best ratio virus:ViroMag R/L

These conditions might require some further optimizations depending on your cells, virus types, MOI, etc.

* Please refer to the following section "Important Notes"

IMPORTANT NOTES – Before you begin

- ✓ For cell lines, seed the cells 24h before transfection in a 96-well plate, 24-well plate or 6-well plate in respectively 150 µL, 400 µL and 2 mL of complete culture medium.
- ✓ For more information on cell in suspension - such as primary T cells - please refer to the following papers
  - *Sacha JB et al. Nat Protoc. 2010 Feb;5(2):239-46. doi: 10.1038/nprot.2009.227*
  - *Barsov EV. Methods Mol Biol. 2009;511:143-58. doi: 10.1007/978-1-59745-447-6_6*
- ✓ Allow reagents to reach RT and gently vortex them before use.
- ✓ Adapt the MOI depending on the viral vector and the type of cells used. MOI can usually vary from 0.5 up to 100.
- ✓ Virus Preparation. Medium or buffer without serum & supplement must be used for the dilution of the virus and the preparation of the virus/ViroMag RLcomplexes. Culture media such as DMEM or OptiMEM or salt-containing buffers such as HBS or PBS are recommended. Alternatively, you can directly use an aliquot of the culture supernatant from a producer cell line.
- ✓ For doses of ViroMag RL less than 1µL, dilute the reagent exclusively with deionized water.
- ✓ We recommend respecting the order of addition of reagents: add the virus suspension to the ViroMag RL tube.
- ✓ For most cell types, a medium change is not required after Magnetofection. However, it may be necessary for some cells that are sensitive to serum/supplement concentration or for transduction synchronization. This can be done immediately after the 20min. incubation on the magnetic plate while keeping the cells onto the magnetic device, or 4 to 6 hours post-Magnetofection.

IMPORTANT NOTES

- Do not freeze the magnetic nanoparticles!
- Polybrene or other additives must NOT be used in combination with ViroMag RL
- **The suggested volume of ViroMag RL is related to infectious particles and not physical viral particles.** ViroMag RL is designed to enhance infection efficiency, thus it is recommended to start with low MOI from 0.5 to 10 with several ViroMag RL volumes.

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

Any questions?

Package content	RL40100: 100 µL of ViroMag RL RL40200: 200 µL of ViroMag RL RL41000: 1mL of ViroMag RL KC30700: ViroMag R/L Starting Kit - 200 µL of ViroMag RL reagent + Super Magnetic Plate
Shipping conditions	Room Temperature
Storage conditions	Store the ViroMag RL reagent at +4°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled.
Product description	ViroMag RL is a magnetic nanoparticles formulation optimized for Retroviruses and Lentiviruses.
Important notice	For research use only. Not for use in diagnostic procedures.

1. Cells preparation

It is recommended to seed or plate the cells the day prior transduction, however cells can also be prepared few hours before the transduction. The suitable cell density will depend on the growth rate and the condition of the cells. Best results are achieved if cells are at least 60-80 % confluent at the time of Magnetofection (refer to Table 1). Cells should be plated in the same manner as required for standard viral gene delivery. For example, the confluency must be low for retroviral vectors, which require cell division for infection.

Culture vessel	Number of adherent cells	Final Transduction Volume*
96-well	0.05 – 0.15 x 10^5	150 µL
24-well	0.5 – 1 x 10^5	500 µL
6-well	2 – 5 x 10^5	2 mL

*Transduction volume corresponds to the volume of culture medium covering the cells plus the volume of the ViroMag RL/viral particles mixture.

Table 1: Recommended adherent cell number to seed per well 24h before transduction experiment

2. Viral particles/ViroMag RL complexes preparation

a. *ViroMag RL*: Vortex the reagent and place the appropriate amounts (refer to Table 2) in an empty microtube.

Culture Vessel	ViroMag RL Quantity (µL)	Suggested ViroMag RL Quantity (µL)	Volume of ViroMag RL / virus solution	Final Transduction Volume*
96 well	0.2 – 3	1.5	50 µL	150 µL
24 well	1 - 12	6	100 µL	500 µL
6 well	5 - 60	30	200 µL	2 mL

*Transduction volume corresponds to the volume of culture medium covering the cells plus the volume of the ViroMag/virus mixture

Table2: Recommended amounts of ViroMag RL, volume of vector preparation and final transduction volume

b. *Viral particles solution*: Add your virus suspension to the tube containing ViroMag RL and mix immediately by pipetting up & down.

NOTE: Prefer virus solutions made in serum-free medium or salt-containing buffers.

c. Incubate at room temperature for 15 to 20 minutes.

3. Transduction

a. Add the ViroMag RL / Virus complexes onto cells drop by drop and gently rock the plate to ensure a uniform distribution.

b. Place the cell culture plate on the magnetic plate during 30 minutes.

c. Remove the magnetic plate.

d. Cultivate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of transgene expression.

NOTE: In case of cells very sensitive to transduction or infection, the medium can be changed after 3-4 hours or 24 hours incubation with fresh medium.

1. Cells preparation

Suspension cells should be prepared in the adequate vessel just before the infection (see Table 3).

Culture vessel	Number of suspension cells	Final Transduction Volume*
96-well	0.5 – 1 x 10 ^{5}	150 µL
24-well	2 – 5 x 10 ^{5}	500 µL
6-well	1 – 2 x 10 ^{6}	2 mL

*Transduction volume corresponds to the volume of culture medium covering the cells plus the volume of the ViroMag RL/viral particles mixture.

Table 3: Recommended suspension cell number to seed per well prior transduction experiment

2. Viral particles/ViroMag RL complexes preparation

a. *ViroMag RL*: Vortex the reagent and place the appropriate amounts (refer to Table 2) in an empty microtube.

b. *Viral particles solution*: Add your virus suspension to the tube containing ViroMag RL and mix immediately by pipetting up & down.

NOTE: Prefer virus solutions made in serum-free medium or salt-containing buffers.

c. Incubate at room temperature for 15 to 20 minutes.

3. Transduction

a. While the ViroMag RL / virus mixtures incubate, prepare the cells to be transduced (as suggested in Table 3). For example, dilute the cells to 5 x 10^5 - 1 x 10^6 / mL in medium (with or without serum- or supplement; depending on cell type and sensitivity of cells towards serum-free conditions) and perform one of the following three options to sediment the cells at the bottom of the culture dish in order to promote the contact with the magnetic nanoparticles.

- Seed the cells on polylysine-coated plates and use the protocol for adherent cells, **OR**
- Briefly, centrifuge the cells (2 minutes) to pellet them and use the protocol for adherent cells, **OR**
- Mix cell suspension with 20-30 µL of CombiMag reagent (Magnetofection) per 1 ml of cell suspension and incubate for 10 - 15 minutes. Then, distribute the cells to your tissue culture dish placed upon the magnetic plate and incubate for 15 more minutes

b. Add the resulting mixture of ViroMag RL/ virus to the cells while keeping the cell culture plate on the magnetic plate.

c. Continue to incubate for 15 minutes.

d. Remove culture plate from magnetic plate.

e. Continue to cultivate cells as desired until evaluation of the transduction experiment.

NOTE: refer to the following papers for a detailed protocol for transduction and synchronous infection of suspension cell (T lymphocyte):

- *Sacha JB et al. Nat Protoc. 2010 Feb;5(2):239-46. doi: 10.1038/nprot.2009.227*
- *Barsov EV. Methods Mol Biol. 2009;511:143-58. doi: 10.1007/978-1-59745-447-6_6.*

Optimization Protocol

In order to get the best out of ViroMag RL several parameters can be optimized:

- ViroMag RL dose & Ratio to viral particles
- cell density and incubation times

1) Start by optimizing the ViroMag RL dose with a fixed MOI. This will vary the concentration of ViroMag RL and the ratio ViroMag RL / Virus. To this end, vary the amount of ViroMag RL in the range suggested in the Table 2. For instance, from 0.2 to 3µL of ViroMag RL in a 96-well plate.

2) Next, use a fixed volume of reagent and vary the MOI.

3) Finally, you can optimize the cell number (density), kinetics of readout and also the incubation time for the magnetofection procedure.

Additional products for Viral Transduction Enhancement

- **ViroMag** for enhancing viral transduction efficiency (suitable for all viruses)
- **AdenoMag** specific for Adenoviral and AAV transduction

Additional products for Virus Capture and Concentration

- **Mag4C-LV** for Lentiviruses
- **Mag4C-AD** for Adenoviruses

Purchaser Notification

Limited License

The purchase of the ViroMag R/L 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 transduction of cells with viruses 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 ViroMag R/L 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 ViroMag R/L kit reagents and documentation to OZ Biosciences, or by destroying all ViroMag R/L components. Purchasers are advised to contact OZ Biosciences with the notification that a ViroMag R/L 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 ViroMag R/L 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

ViroMag R/L 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.

EUROPE & ASIA
OZ Biosciences SAS

163 avenue de Luminy
Case 922, zone entreprise
13288 Marseille cedex 09
France

Ph: +33 (0) 486 948 516
Fax: +33 (0) 463 740 015

contact@ozbiosciences.com
order@ozbiosciences.com
tech@ozbiosciences.com

USA & CANADA
OZ Biosciences INC

7975 Dunbrook Road
Suite B
San Diego CA 92126
USA

Ph: + 1-858-246-7840
Fax: + 1-855-631-0626

contactUSA@ozbiosciences.com
orderUSA@ozbiosciences.com
techUSA@ozbiosciences.com

Rev. 04/2025