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Viral Applications

ViroMag™

Transduction reagent

Enhance viral transduction efficiency

Protocol

Magnetofection Technology

This reagent needs to be used with a magnetic plate

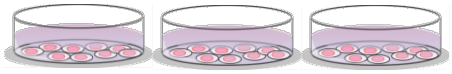
OZBIOSCIENCES

The art of delivery systems

ViroMag Quick Protocol

To find the ideal conditions, **ViroMag** 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

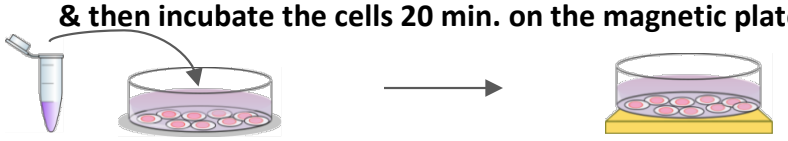
3 **Prepare 4 tubes of ViroMag (with different amounts of magnetic beads)***
The volumes of ViroMag to be tested is fixed and is the same for any MOI

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

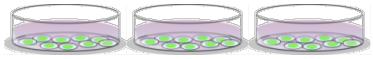
4 **Mix each tube of viral particles (step 2) to each tube of ViroMag (step 3)***

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

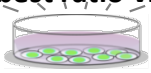
6 **Distribute each mix drop by drop onto the cells to insure uniform distribution & then incubate the cells 20 min. on the magnetic plate**



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**



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 a detailed protocol for transduction and synchronous infection of suspension cells - such as T lymphocyte - 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 complexes. 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 less than 1µL, dilute the reagent exclusively with deionized water.

- ✓ We recommend respecting the order of addition; add the virus suspension to the ViroMag 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 h post-Magnetofection.

IMPORTANT NOTES

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

ViroMag Reagent | Specifications

Package content	VM40100: 100 µL of ViroMag VM40200: 200 µL of ViroMag VM41000: 1mL of ViroMag KC30500: ViroMag Starting Kit - 200 µL of ViroMag reagent + Super Magnetic Plate
Shipping conditions	Room Temperature
Storage conditions	Store the ViroMag reagent at +4°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled.
Product Descriptions	ViroMag is a magnetic nanoparticles formulation dedicated to increase virus infection and transduction capacities. ViroMag is suitable for all kind of viral particles.
Important notice	For research use only. Not for use in diagnostic procedures.

Protocol | Lentiviral transduction enhancement in adherent cells

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 can be high for adenoviral vectors but 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/viral particles mixture.

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

2. Viral particles/ViroMag complexes preparation

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

Culture Vessel	ViroMag Quantity (µL)	Suggested ViroMag Quantity (µL)	Volume of ViroMag/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 volume of vector preparation and final transduction volume

b. *Viral particles solution*: Add your virus suspension to the tube containing ViroMag 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 / 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.

Protocol | Lentiviral transduction enhancement in suspension cells

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/viral particles mixture.

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

2. Viral particles/ViroMag complexes preparation

a. *ViroMag*: 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 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 / virus mixtures incubate, prepare the cells to be transduced (see 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 / 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.

Optimization Protocol

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

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

1) Start by optimizing the ViroMag dose with a fixed MOI. This will vary the concentration of ViroMag and the ratio ViroMag / Virus. To this end, vary the amount of ViroMag in the range suggested in the Table 2. For instance, from 0.2 to 3µL of ViroMag 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 RL** for enhancing Lentiviral and Retroviral transduction efficiency
- **AdenoMag** specific for Adenoviral and AAV transduction

Additional products for Virus Capture and Concentration

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

Purchaser Notification

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