LentiMag™ Reagent & Magnetotransduction Kit

Cat. #s LV800A-1, LV801A-1

User Manual

Store kit at +4°C on receipt

(ver. 2-081211)

Contents

I. Introduction and Background

A. Overview

LentiMag™ reagent has been specifically developed for improving transduction of mammalian cells using retroviral- and lentiviral-based gene vectors. The technology employs magnetic force exerted upon lentiviral vectors associated with magnetic nanoparticles to pull the virus particles towards target cells. LentiMag™ increases transduction efficiency in common cell lines compared to reagents such as Polybrene, and makes possible the transduction of nonpermissive cells (primary cells, stem cells, and difficult-to-transduce cell lines).

The magnetic plate supplied with the kit has been designed to exert the magnetic field on lentiviral vectors complexed with LentiMag™ nanoparticles, and is compatible with most standard cell culture dishes (T-25 and T-75 flasks, 60 and 100 mm diameter dishes, and 6-, 12-, 24-, 48-, and 96-well plates).

In this handbook we provide two protocols: one for adherent cells, and one for cells grown in suspension. An overview of the procedure is shown in Figure 1 below.

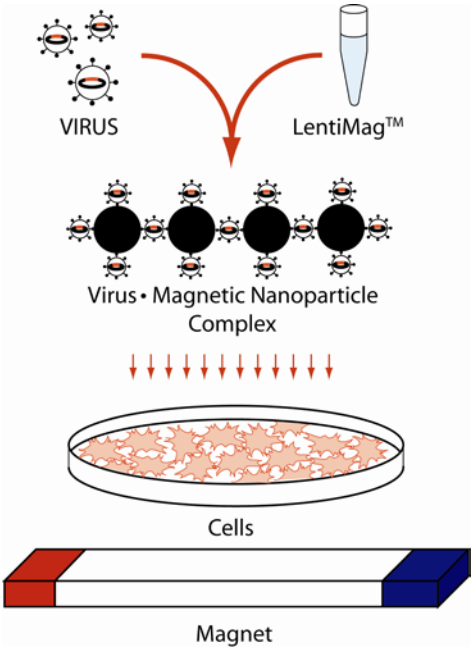

Fig. 1. Overview of LentiMag™ Magnetotransduction

B. Background

The rate at which lentiviral vectors bind to and infect target cells (transduction) is predominantly controlled by diffusion (7). Only that fraction of lentiviral vectors that contact target cells via Brownian movement within a given incubation time can bind to and transduce the target cells (2). To overcome these limitations, magnetic nanoparticles have been exploited to capture lentiviral vectors, and attract the vectors to target cells by use of an external magnetic field (8). These approaches have greatly improved transduction efficiencies of lentivrial vectors (1-12). System Biosciences, in an attempt to provide the greatest lentiviral transduction efficiencies to our customers, have developed a magnetic nanoparticle suspension, LentiMag™, that effectively binds our HIV- and FIV-based vectors and very quickly concentrates them onto target cells by use of a magnetic plate. Using LentiMag™, higher transduction efficiencies have been achieved compared to transductions performed with Polybrene (hexadimethrine bromide). Figures 2a and 2b show typical results obtained with LentiMag™ versus Polybrene for various cell types.

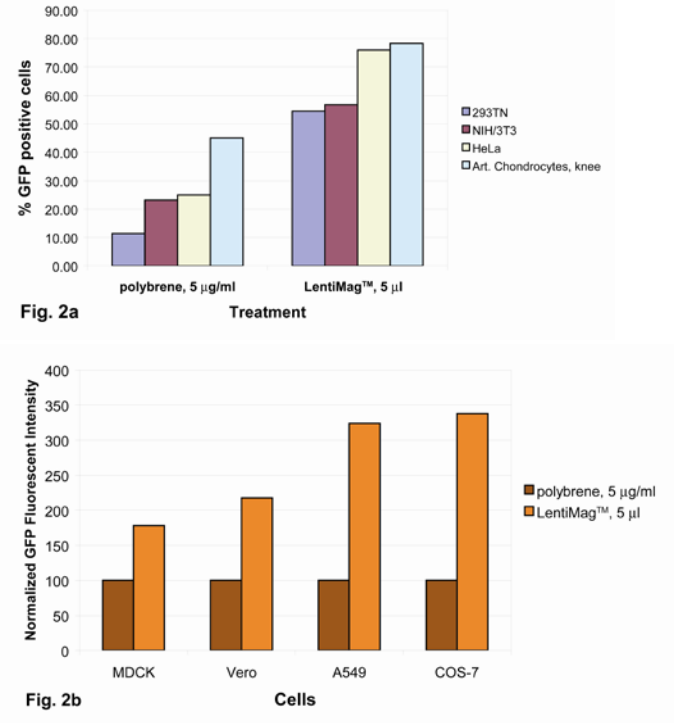

Fig. 2. Comparison of Target Cells Transduced with Polybrene® vs. LentiMag™

C. List of Components

LentiMag™ Magnetotransduction kits (Cat. # LV800A-1) contain the following components:

- 1 tube containing 50 μl of LentiMag™ Reagent, sufficient for 10 transductions in a 24-well plate format.
- 1 magnetic plate

LentiMag™ Reagent (Cat. # LV801A-1) can also be purchased separately

- 1 tube containing 50 μl LentiMag™, sufficient for 10 transductions in a 24-well plate format

Stability and Storage

Storage: +4°C
Upon receipt and for long-term storage, keep LentiMag™ at +4°C. Magnetotransduction kits are stable for at least one year at the recommended storage temperature.

- **DO NOT FREEZE THE MAGNETIC NANOPARTICLES!**
- **DO NOT ADD ANYTHING TO THE STOCK SOLUTION OF MAGNETIC NANOPARTICLES!**
- **MAGNETIC PLATE WILL STICK TIGHTLY TO METAL SURFACES. AVOID USE ON METAL TABLES OR OTHER METAL SURFACES!**

Quality Control

To assure the performance of each lot of LentiMag™ produced, each lot is qualified using rigorous standards. *In vitro* assays are conducted to test the quality and activity of each kit component.

Components	Standard Quality Controls
LentiMag™	1) Quality and size homogeneity of the magnetic nanoparticles. 2) Stability of the magnetic nanoparticles formulations. 3) LentiMag™ transduction efficacies with a recombinant VSV-G-pseudotyped HIV-based lentiviral vector (GFP reporter gene) on HeLa cells. Every lot shall have an acceptance specification of > 80% of the activity of the reference lot. 4) Sterility. Thioglycolate assay: absence of fungal and bacterial contamination shall be obtained for 7 days.
Magnetic Plate	1) Tests of solidity and magnetic field force

Shipping conditions: Room Temperature

II. General Considerations

The instructions given below represent sample protocols that were applied successfully with a variety of cells and lentiviral vectors. Our R&D team has tested and optimized the LentiMag™ reagent in order to provide you with a straightforward and efficient protocol. Therefore, we recommend you start by following our general protocol as a guideline to obtain high quality data quickly, and if necessary, advise optimization of the experimental parameters in order to achieve the best results. Optimal conditions do vary from cell to cell and are highly dependent upon the type of virus used, its titer, the composition of the viral solution, and cell culture conditions. Consequently, the amount, concentration, and ratio of the individual components (virus and LentiMag™), the time course and the number of cells may have to be adjusted to obtain the best results. Several optimization and troubleshooting protocols are available in the Appendix.

A. Safety Guidelines

Use of the LentiMag™ Magnetotransduction kit with HIV- and FIV-based or other lentiviral transduction systems falls within NIH Biosafety Level 2 criteria due to the potential biohazard risk of possible recombination with endogenous retroviral sequences to form self-replicating virus, or the possibility of mutagenesis. For a description of laboratory biosafety level criteria, consult the Centers for Disease Control Office of Health and Safety Web site at http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s3.htm. It is also important to check with the health and safety guidelines at your institution regarding the use of lentiviral vectors, and always follow standard microbiological practices, which include:

- Wearing gloves, safety glasses, and a lab coat at all times when conducting the procedure
- Always working with pseudoviral particles in a Class II laminar flow hood
- Carefully performing all procedures to minimize the creation of aerosols or splashes
- Decontamination of all work surfaces at least once a day and immediately after working with lentiviral particles
- Decontamination of all cultures, viral stocks, and other regulated wastes before disposal by an approved method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory area are to be placed in a durable, leakproof, properly marked (“Biohazard”, “infectious waste”) container and sealed for transportation from the laboratory.

B. Cell Culture

It is recommended to seed the cells the day prior to transduction. Suspension cells should be prepared in the adequate vessel just before the transduction (see below for specific protocol). The suitable cell density will depend on the growth rate and the condition of the cells. Best results are achieved if adherent cells are at least 60-80% confluent at the time of Magnetotransduction. Suggested cell numbers for seeding specific cell culture vessels are shown in Table 1.

Culture vessel	Number of adherent cells	Number of suspension cells	Final Transduction Volume*
96-well	$0.05 – 0.15 \times 10^5$	$0.5 – 1 \times 10^5$	150 µL
24-well	$0.5 – 1 \times 10^5$	$2 – 5 \times 10^5$	500 µL
12-well	$1 – 2 \times 10^5$	$2.5 – 10 \times 10^5$	1 mL
6-well	$2 – 5 \times 10^5$	$1 – 2 \times 10^6$	2 mL
60 mm dish	$5 – 10 \times 10^5$	$2.5 – 5 \times 10^6$	4 mL
90 – 100 mm dish	$15 – 30 \times 10^5$	$5 – 10 \times 10^6$	8 mL
T-25 flask	$5 – 10 \times 10^5$	$2.5 – 5 \times 10^6$	5 mL
T-75 flask	$20 – 50 \times 10^5$	$5 – 15 \times 10^6$	10 mL

Table 1. Recommended cell number.

* Transduction volume corresponds to the volume of culture medium covering the cells plus the volume of the LentiMag™ / virus mixture.

III. Protocol

A. Adherent Cells

Use the following protocol to transduce **adherent cells** with LentiMag™ in a **12-well format**. For other formats, see **Tables 1 and 2**. All amounts are given on a per well basis.

1) Plate 1-2 × 10^5 cells the day before magnetotransduction in 0.9 ml growth medium. Incubate overnight. Inspect cells prior to magnetotransduction to ensure they are at the proper confluency (60-80%).

2) Add 12 µl LentiMag™ to a **sterile** 1.5 ml microcentrifuge tube(s).

3) Add your virus preparation to the tube(s) containing LentiMag™ and mix immediately by pipetting. Virus preparation is preferably in serum-free medium or salt-containing buffers (e.g., PBS).

4) Dilute LentiMag™ / virus solution to 100 µl using serum-free medium or PBS.

5) Incubate 5 to 15 minutes either at room temperature or on ice.

6) Dropwise, add the LentiMag™ / virus mixture to the cells to be transduced.

7) Place the cell culture vessel upon the magnetic plate for 15 minutes. Longer incubation times (30 or 60 minutes) can also be used.

8) Remove the magnetic plate and cultivate the cells under standard conditions until evaluation of the transduction experiment. Optionally perform a medium change the following day.

Culture Vessel	LentiMag™ Quantity (µL)	Volume of LentiMag™/virus solution	Final Transduction Volume*
96 well	1.5	50 µL	150 µL
24 well	5	100 µL	500 µL
12 well	12	100 µL	1 mL
6 well	30	200 µL	2 mL
60 mm dish	60	400 µL	4 mL
90 - 100 mm dish	150	800 µL	8 mL
T-25 flask	60	500 µL	5 mL
T-75 flask	150	1000 µL	10 mL

Table 2: Recommended amount of LentiMag™, volumes of vector preparation, and final transduction volume.

B. Suspension Cells

Use the following protocol to transduce **suspension cells** with LentiMag™ in a **12-well format**. For other formats, see **Tables 1 and 2**. All amounts are given on a per well basis.

1) Seed 2.5-10 × 10^5 cells the day before magnetotransduction in 0.9 ml growth medium. Incubate overnight.
   Note: suspension cells can be adhered to the bottom of the tissue culture plate if polylysine-coated plates are used. Alternatively, cells can be briefly centrifuged for 2 min. to pellet the cells to the bottom of the plate using a microplate rotor or adaptor for microplates.

2) Add 12 µl LentiMag™ to a **sterile** 1.5 ml microcentrifuge tube(s).

3) Add your virus preparation to the tube(s) containing LentiMag™ and mix immediately by pipetting. Virus preparation is preferably in serum-free medium or salt-containing buffers (e.g., PBS).

4) Dilute LentiMag™ / virus solution to 100 µl using serum-free medium or PBS.

5) Incubate 5 to 15 minutes either at room temperature or on ice.

6) Add the resulting mixture of LentiMag™ / virus to the cells while keeping the cell culture dish on the magnetic plate.

7) Continue to incubate for 15 minutes.

8) Remove culture dish from magnetic plate.

9) Cultivate cells until evaluation of the transduction experiment.

IV. References

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. **Plank C, et al.** Enhancing and targeting nucleic acid delivery by magnetic force. *Expert Opin Biol Ther.* 2003; 3(5):745-58.

3. **Schillinger, U., et al.** Advances in Magnetofection – magnetically guided nucleic acid delivery. 2005. *J. Magn. Magn. Mat.* **293**: 501-508.

4. **Mok, H., et al.** Evaluation of polyethylene glycol modification of first-generation and helper-dependent adenoviral vectors to reduce innate immune responses. 2005. *Mol. Ther.* **11**(1): 66-79.

5. **Pandori, M.W., et al.** Adenovirus-Microbead Conjugates Possess Enhanced Infectivity: A New Strategy to Localized Gene Delivery. 2002. *Virology* **299**: 204-212.

6. **Mah, C., et al.** Improved Method of Recombinant AAV2 Delivery for Systemic Targeted Gene Therapy. 2002. *Mol. Ther.* **6**(1): 106-112.

V. Appendix

A. Critical Parameters for Best Performance

1) Cell culture conditions. Best results are achieved when cells are 60 to 80% confluent at the time of the magnetotransduction. If necessary, you can remove the culture medium containing the transduction mixture by washing after 8-24 hours, and replace it with fresh medium.

2) LentiMag™ quantity. We often observed good effects at very low doses of LentiMag™ (2-3 µL / well for a 24-well plate). However, the efficiency may depend on the cell line and virus type used. Consequently, we suggest you start by testing a range of LentiMag™ volumes in order to obtain the best experimental conditions. See Section B below, Protocol Optimization and Troubleshooting.

3) Incubation time on magnetic plate. The infection time depends on the amount/concentration of virus used. Indeed, longer incubation under the magnetic field is required with very low viral titers, whereas with high viral doses short incubation times are sufficient.

B. Protocol Optimization and Troubleshooting

In order to get the best results from LentiMag™, several parameters can be optimized:

- LentiMag™ dose and ratio of LentiMag™ to virus
- Cell type, cell density and incubation times

The System Biosciences team has investigated numerous factors during the course of the R&D program of LentiMag™. Based on our experience, we recommend starting from the experimental protocol described above (section III) and optimize one parameter at a time.

1) Start by optimizing the LentiMag™ dose with a **fixed amount of virus**. This will vary the concentration of LentiMag™ and the ratio LentiMag™ **/** virus. Vary the amount of LentiMag™ in the range suggested in the **Table 4**. For instance, from 0.2 to 3 µL of LentiMag™ in a 96-well plate.

Culture Vessel	ViroMag Range (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	5	100 µL	500 µL
12 well	2 - 24	12	100 µL	1 mL
6 well	5 - 60	30	200 µL	2 mL
60 mm dish	10 - 120	60	400 µL	4 mL
90 - 100 mm dish	30 - 300	150	800 µL	8 mL
T-25 flask	10 - 120	60	500 µL	5 mL
T-75 flask	30 - 300	150	1000 µL	10 mL

Table 4: Range of LentiMag™ quantities for different tissue culture vessels.

2) Next, you can inverse the procedure by optimizing the dose of virus with a **fixed amount of LentiMag™ reagent**.

3) After having identified the correct quantity of LentiMag™ and virus, optimize the **cell number** (density) and **time course of incubation**, between LentiMag™ and virus and time of incubation on the magnetic plate.

C. Related Products

- **Lentivector Packaging Kits**

  For FIV-based Vectors: pPACKF1™ (Cat. # LV100A-1)

  For HIV-based Vectors: pPACKH1™ (Cat. # LV500A-1)

  Unique plasmid mixes that produce all the necessary viral proteins and the VSV-G envelope glycoprotein from vesicular stomatitis virus required to make active pseudoviral particles. Producer Cell Line 293TN (SBI Cat. # LV900A-1) transiently transfected with the packaging plasmids and an HIV-based lentiviral construct produce packaged viral particles containing the lentiviral construct of interest.

- **293TN Human Kidney Producer Cell Line** (SBI, Cat. # LV900A-1)
  For packaging of plasmid lentivector constructs.

- **Lentivector Rapid Titer PCR Kit** (Cat. # LV950A-1 [for human cells], LV951A-1 [for mouse cells])
  Allows you to measure copy number (MOI) of integrated lentiviral constructs in genomic DNA of target cells after transduction with any of SBI's FIV or HIV-based lentivectors.

- **Packaged Positive Transduction Controls**

  FIV-based: pSIF1-H1-siLuc-copGFP (Cat. # LV201B-1)

  HIV-based: pSIH1-copGFP (Cat. # LV600A-1)

  Packaged Positive control lentivectors allow you to measure transduction efficiency in target cells based on percent of GFP-positive cells. The H1-siLuc lentivector expresses an siRNA targeting Luciferase.

- **shRNA Cloning and Expression Lentivectors** (many)
  These FIV and HIV-based single-promoter shRNA cloning vectors allow you to clone and express shRNA constructs. For a list of currently available vectors, please visit our website at www.systembio.com.

- **cDNA Cloning and Expression Lentivectors** (many)
  These FIV and HIV-based cDNA cloning vectors allow strong and ubiquitous expression of your gene of interest. Choose from copGFP or puromycin selection markers. For a list of currently available vectors, please visit our website at www.systembio.com.

- **PathNet™ Transcriptional Reporter Lentivectors** (many)
  Detect the activation of transcriptional factors (TFs) in a natural chromosomal environment based on a lentivector reporter construct and create stable cell lines. Available in plasmid form or pre-packaged in pseudoviral particles. Choose from copGFP, Luciferase, or β-Gal reporters. For a list of currently available vectors, please visit our website at www.systembio.com.

- **GeneNet™ siRNA Libraries**
  Identify whole sets of genes functionally responsible for any selectable phenotype in a single genome-wide screening. Available in pre-packaged for plasmid form. For a list of currently available siRNA Libraries, please visit our website at www.systembio.com.

D. Technical Support

For more information about SBI products and to download manuals in PDF format, please visit our web site:

http://www.systembio.com

For additional information or technical assistance, please call or email us at:

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Technical Support: tech@systembio.com
Ordering Information: orders@systembio.com

VI. Licensing and Warranty Statement

Limited Use License

Use of the LentiMag™ Reagent and LentiMag™ Magnetotransduction Kit (*i.e.*, the "Product") is subject to the following terms and conditions. If the terms and conditions are not acceptable, return all components of the Product to System Biosciences (SBI) within 7 calendar days. Purchase and use of any part of the Product constitutes acceptance of the above terms.

The purchaser of the Product is granted a limited license to use the Product under the following terms and conditions:

The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use.

The Product may not be resold, modified for resale, or used to manufacture commercial products without prior written consent of SBI.

This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

SBI has pending patent applications related to the Product. For information concerning licenses for commercial use, contact SBI.

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