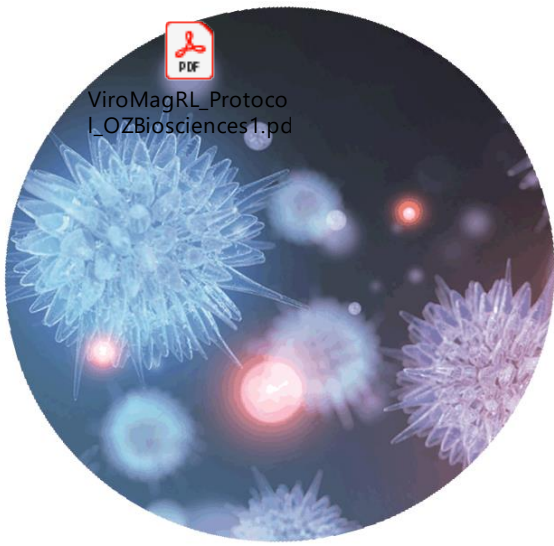




ViroMagRL_Protocol_OZBiosciences1.pdf



Viral Applications

M ViroMag Stem

Transduction enhancer reagent
Lentiviral Transduction of Stem cells

Protocol



Magnetofection Technology

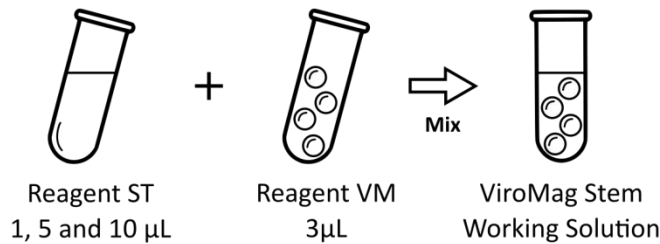
This reagent needs to be used with a magnetic plate

ViroMag Stem - Quick Protocol

Use the following protocol to find the ideal conditions for ViroMag Stem in **24-well plate**. Adjust volumes to different well formats according to Table 1. If the lentiviral transduction/infection conditions are unknown, we recommend starting with a MOI of 2 using a lentiviral vector encoding for a fluorescent protein.

ViroMag STEM Working Solution

Before Beginning, prepare 3 ViroMag Stem Working Solutions (WS) by mixing 1, 5 or 10 μL Reagent ST with 3 μL Reagent VM

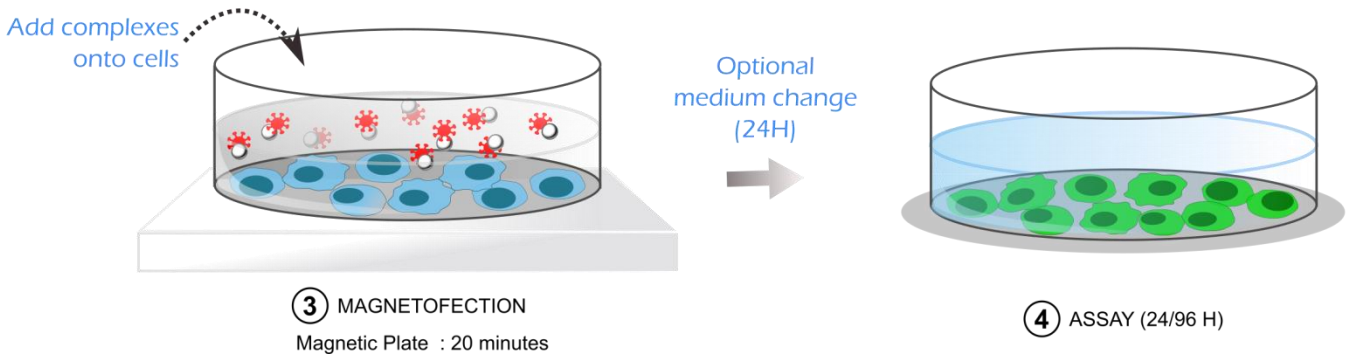
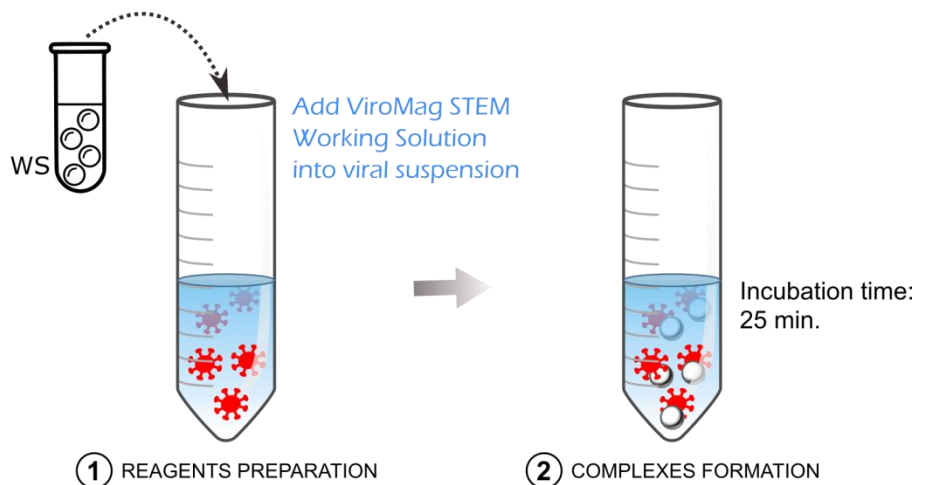


	ViroMag Stem working solution	
	Reagent ST	Reagent VM
Tube 1	-	-
Tube 2	1 μL	3 μL
Tube 3	5 μL	3 μL
Tube 4	10 μL	3 μL

Viral solution

Dilute virus into culture medium: sufficient for 4 samples (50 μL each).

MOI 2 is recommended in case of unknown lentiviral transduction conditions.



IMPORTANT NOTES – Before you begin

- ✓ Allow reagents to reach room temperature before starting
- ✓ Do not change the transduction conditions already settled: simply add the ViroMag Stem to your established infection/transduction protocol
- ✓ Do not mix Reagent ST or Reagent VM with any other viral enhancer or chemicals
- ✓ If transduction conditions are unknown, we recommend beginning with a MOI of 2
- ✓ For cell culture transduction assays, we suggest to first test 3 dilutions (1:500, 1:100 and 1:50) of Reagent ST with 3µL or Reagent VM.
- ✓ Refer to optimization protocol (paragraph 2.5) for gaining more information on how to optimize conditions.

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



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Any questions?



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ViroMag Stem Transduction Enhancer | Specifications

Package content	VMST100: ViroMag Stem 100 transductions (500 µL of Reagent ST + 300 µL of Reagent VM) VMST300: ViroMag Stem 300 transductions (1500 µL of Reagent ST + 900 µL of Reagent VM) KC31000: 1 Super Magnetic plate + Reagent ST 500 µL + Reagent VM 300 µL
Shipping condition	Room Temperature
Storage conditions	Upon reception and for long-term use, store Reagent ST at -20°C and Reagent VM at +4°C.
Shelf life	1 year from the date of purchase when properly stored and handled
Product description	ViroMag Stem , a Magnetofection™-based Lentiviral Transduction Enhancer, enables improved viral driven genetic modification in a wide range of stem cells including CD34+ Hematopoietic Stem Cell.
Important notice	For research use only. Not for use in diagnostic procedures

1. Cells Preparation

Cell culture prior to transduction: the day before transduction prepare the cells according to the table below.

Effects of lentiviral transduction are generally observed after 48 to 96 h incubation. Cells should be 20-50 % confluent at the time of transduction (see the suggested cell number in the **Table 1**).

Tissue culture dish format	Surface area per well ¹	Cell number	Recommended volume for culture	Recommended volumes for viral preparation
96-well plate	0.3 cm ²	3 - 8 x 1.10 ³	100 µL	50 µL
24-well plate	2 cm ²	2 - 4 x 1.10 ⁴	500 µL	50 µL
12-well plate	4 cm ²	4 - 8 x 1.10 ⁴	1 mL	100 µL
6-well plate	9 cm ²	1 - 2 x 1.10 ⁵	2 mL	200 µL

¹ Surfaces area may vary depending on the manufacturer.

Table 1: Recommended cell number and volume for viral/ViroMag Stem complexes preparation

2. Optimization Protocol

Depending on cell type (primary/cell lines), viral particles, gene of interest, culture conditions, readout..., transduction or infection efficiencies may vary. It may be necessary to optimize the conditions by (1) varying volumes of each tube composing ViroMag Stem, (2) tuning finely transduction parameters or (3) centrifuging the cells.

NOTE: do not change the MOI that you usually use. However, when the infection capacity of the virus is unknown, we recommend starting with a MOI of 2 (2 viral particles per cell).

- a. Optimizing ViroMag Stem volume: using a fixed MOI, vary the volume of each tubes composing ViroMag Stem working solution.
- b. Finely tuning transduction parameters: we have observed that replacing culture medium at the time of transduction with complete medium containing viral particles/ViroMag Stem could improve the overall efficiency.
- c. Optionally perform a medium change after 24H, and incubate 24H to 96H
- d. Raising efficiency using Centrifugation step: depending on the cell type and the application, a centrifugation step can be added to the standard protocol. Cells are prepared the day of transduction, counted, pelleted and suspended in Lentivirus/ViroMag Stem mix before centrifugation.

NOTE: Centrifugation may negatively influence cell viability.

NOTES

Additional products for Viral Transduction Enhancement

- **ViroMag** for enhancing viral transduction efficiency (suitable for all viruses)
- **ViroMag R/L** for enhancing Lentiviral and Retroviral transduction efficiency
- **AdenoMag** specific for Adenoviral and AAV transduction

Additional products for Virus Production

- **Helix-IN DNA Transfection Reagent**

Additional products for Virus Capture and Concentration

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

Purchaser Notification

Limited License

The purchase of the ViroMag Stem Premium reagent grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in section 1, Kit Contents) for the sole purpose of in-house research only, provided that no license, right or permission is granted hereunder to a non-academic, for-profit or commercial Licensee to use the ViroMag Stem Premium reagent for *ex vivo* gene therapy for hemoglobinopathies. The license does not include the use for any commercial or development purpose, including but not limited to any use for a) manufacturing, production, quality control, b) providing services, information or data, c) therapeutic, diagnostic, vaccine or prophylactic purposes or d) any applications which require regulatory approval as well as e) any clinical activities *in vivo* or *ex vivo*. The licensed use is limited to 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.

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Product Use Limitations

The ViroMag Stem Premium reagent 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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