3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

MISSION® Magnetic Transduction Reagents

Catalog Numbers: **SHM01, SHM02, SHM03, SHM04, SHM05**
Storage Temperature: 2-8 °C for ExpressMag beads, room temperature storage for magnets

TECHNICAL BULLETIN

Product Description

Small interfering RNAs (siRNAs) expressed from short hairpin RNAs (shRNAs) are a powerful way to mediate gene specific RNA interference (RNAi) in mammalian cells. The MISSION product line is a lentiviral-vector based RNAi library targeting annotated mouse and human genes. Amphotropic lentiviral particles deliver shRNA that generate intracellular siRNA, allowing for screening in a wide range of mammalian cell types. In these cells, MISSION shRNA libraries and clones permit rapid, cost efficient loss-of-function and genetic interaction screens.

The ExpressMag® technology has been specifically designed to improve infection of cells using viral-based vectors. MISSION lentiviruses couple to magnetic nanoparticles, and magnetic force pulls the nanoparticles towards the cells to be transduced. This concentrates virus near the cell, resulting in: transduction at higher efficiencies, transduction of hard-to-transduce cell types (primary cells, stem cells, T-cells, etc.), reduction of transduction time, and reduction of initial viral load.

Precautions and Disclaimers

These products are for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Magnets should be kept away from magnetic storage devices, such as hard disks, ID cards, and credit cards, and from electronic devices and ferromagnetic materials. Persons with cardiac pacemakers should not work with these magnets.

Storage/Stability

The ExpressMag Beads are guaranteed to be stable for at least 2 years when stored at 2-8 °C. Magnetic plates should be stored at room temperature.
Do not freeze the magnetic nanoparticles.

Reagents - The ExpressMag products are provided in multiple volumes and formats:

Catalog Number	Package Size	Description	Number of Transductions in a 96-well plate 1.5 µL ExpressMag Beads/well
SHM01	Kit	MISSION ExpressMag Super Magnetic Kit Super Mag Plate and 100 µL ExpressMag Beads	63
SHM02	Kit	MISSION ExpressMag 96-well Magnetic Kit 96-well ExpressMag Plate and 100 µL ExpressMag Beads	63
SHM03	100 µL	MISSION ExpressMag Beads	63
SHM03	300 µL	MISSION ExpressMag Beads	190
SHM03	1 mL	MISSION ExpressMag Beads	633
SHM04	1 each	MISSION ExpressMag Super Magnetic Plate	N/A
SHM05	1 each	MISSION ExpressMag 96-well Magnet	N/A

Overview of Magnetic Transduction Variables
The ExpressMag Beads are fully compatible with a variety of standard cell culture dishes.

Table 1: Recommended seeding densities and reaction volumes

Plate Or Vessel	Adherent Cell Numbers	Suspension Cell Numbers	Recommended Volume of ExpressMag Beads (μL)	Volume of ExpressMag Beads and Virus Solution (μL)	Final Transduction Volume (ml)
96 well	0.5-1.5 x 10^4	0.5-1 x 10^5	1.5	50	0.15
24 well	0.5-1 x 10^5	2-5 x 10^5	6	100	0.5
12 well	1-2 x 10^5	2.5-10 x 10^5	12	100	1.0
6 well	2-5 x 10^5	1-2 x 10^6	30	200	2.0
60 mm dish	5-10 x 10^5	2.5-5 x 10^6	60	400	4.0
90-100 mm dish	15-30 x 10^5	5-10 x 10^6	150	800	8.0
T-25 flask	5-10 x 10^5	2.5-5 x 10^6	60	500	5.0
T-75 flask	20-50 x 10^5	5-15 x 10^6	150	1000	10.0

Protocols

Magnetic Transduction of Adherent Cells

Day 1
1. Plate cells in your desired tissue culture format as suggested in Table 1.

Day 2
2. In a separate tube or plate, add the desired volume of ExpressMag Beads using the recommended ranges from Table 1 as a guideline. It is important to optimize this volume in your conditions, as cells demonstrate varying sensitivities to the product.
   Note: If required, the ExpressMag Beads may be diluted in de-ionized water only.
3. Add your virus preparation to the ExpressMag - containing tube, bringing the total volume to the amount indicated in Table 1.
4. Mix well by immediately pipetting up and down.
5. Incubate the ExpressMag and virus cocktail at room temperature for 15 minutes to allow conjugation to occur.
6. Add the cocktail to the cells to be transduced. The total volume of media, Beads, and virus solution is indicated in the last column of Table 1.
7. Place the culture vessel upon the magnetic plate for 15 minutes at room temperature. The incubation may be shortened to 1 to 5 minutes to achieve synchronicity of infection.
8. Remove the culture vessel from the magnetic plate and cultivate cells under standard conditions until evaluation of transduction is desired.

Day 3
9. Perform a media change on the cells if desired.

Magnetic Transduction of Cells in Suspension

Day 1

1. Plate cells in your desired tissue culture vessel as suggested in Table 1.

Day 2

2. In a separate tube or plate, add the desired volume of ExpressMag Beads using the recommended ranges from Table 1 as a guideline. It is important to optimize this volume in your conditions, as cells demonstrate varying sensitivities to the product.
   Note: If required, the ExpressMag Beads may be diluted in deionized water only.
3. Add your virus preparation to the ExpressMag-containing vessel and mix well by immediately pipetting up and down.
4. Incubate the ExpressMag and virus cocktail at room temperature for 15 minutes to allow conjugation to occur.
5. During the incubation in step 4, prepare the cells to be transduced. Dilute the cells according to the guidelines in Table 1 in select medium. Depending on the cell type and sensitivity to serum-free conditions, this step may be done in either the presence or absence of serum and additional supplements.
6. Select one of the following options to pellet the cells at the bottom of the final culture vessel in order to facilitate contact with the magnetic nanoparticles.
   a. Seed the cells on SigmaScreen™ Poly-D-Lysine coated plates, Catalog Number Z382493, and use the protocol for adherent cells, or
   b. Briefly centrifuge the cells at 1000 rpm for 2 minutes and follow the adherent protocol
7. Once the cells are pelleted and the incubation is complete, add the ExpressMag and virus cocktail to the cells and place on magnetic plate.
8. Incubate culture vessel on magnetic plate for 15 minutes at room temperature.
9. Remove the culture plate from the magnetic plate and cultivate cells under standard conditions until evaluation of transduction experiment.

Day 3

10. Perform a media change on the cells if desired.

Optimization of Magnetic Transduction

We highly recommend optimization of the ExpressMag Beads in order to obtain the best results through magnetic transduction. Several parameters should be optimized, including cell density, volume of ExpressMag Beads, amount of MISSION lentiviral particles, ratio of ExpressMag Beads to MISSION lentiviral particles, and incubation times.

MISSION TurboGFP™ Control Transduction Particles, Catalog Number SHC003V, can be used to assess transduction efficiency by visual inspection or flow cytometry and optimize protocols with ExpressMag Beads. The TurboGFP control vector consists of the lentiviral backbone vector, pLKO.1-puro, containing a gene encoding TurboGFP driven by the CMV promoter.

As a negative control, MISSION Non-Target shRNA Control Transduction Particles, Catalog Number SHC002V, should be coupled to ExpressMag Beads. The vector for this control contains an shRNA insert that does not target any known human or mouse gene due to at least 5 base pair mismatches with all known genes in those genomes. This controls for non-specific events during magnetic transduction and activation of the RNAi pathway.

Primary Cells and Immortalized Cell Lines Successfully Transduced with ExpressMag Beads	
Primary Cells	**Cell Lines**
Bone Marrow Macrophages, mouse	A549
GBM	B95a
HUVEC	CHO
Keratinocytes, human	H9
MEF	HeLa
PBL	K562
PBMC	KS-1
T Cells	L929
T Cells CD4+, human	MM-AN
	NIH3T3
	NYGM
	SKOV3
	T98G
	U251
	U373
	U87
	Vero
	YH-13
	YK6-1

Troubleshooting Guide

The critical parameters for magnetic transduction are the cell culture conditions, ratio of ExpressMag Beads to lentivirus, and the incubation times. It is important that these parameters be optimized for the experimental conditions, type of virus, and cell type.

1. Cell Culture Conditions: Best results are achieved when cells are 60-80% confluent at the time of transduction. Suspension cells should be seeded at higher densities since they are not prepared until the time of experimentation.
2. ExpressMag:Lentivirus Ratio: Even though high levels of transduction are often detected at low viral doses, the efficiency is primarily dependent on the cell line used. Optimization experiments to determine the appropriate volume of ExpressMag Beads and MISSION lentivirus should be performed with the recommended values in Table 1 as a guide.
3. Incubation Time: The time course of infection is based on the volume of lentivirus used. Even though infection typically occurs very rapidly, it may be necessary to extend the magnetic exposure up to 1 hour depending on the lentiviral titer. For exposures longer than 15 min, place tissue culture vessel and magnet in incubator.

Cytotoxicity: Lentiviral supernatant may be diluted in serum-free medium or HBSS to lower the concentration of serum and additional supplements. It is also possible to perform a media change on the cells 8-24 hours following transduction to replenish vital nutrients.

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