Transfection reagent

In Vivo ViroMag

In Vivo Transduction

Protocol

Magnetofection Technology
This reagent needs to be used with specific magnets

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The art of delivery systems

IMPORTANT NOTES – Before you begin

1. The conditions provided above might require some further optimizations depending on your nucleic acids, animal, territory, routes of injection etc…
2. We suggest to use 10 to 20 µL of *In vivo* ViroMag per 1x106 infectious viral particles.
3. Allow reagents to reach RT and gently vortex them before forming complexes.
4. The final DNA concentration should not exceed 0.5 mg/mL.
5. Dilutes the reagents with deionized water for doses less than 1µL. Discard the diluted reagent after use.
6. Nucleic acids should be as pure as possible, endotoxins free and prepare in water
7. For the complexes preparation and injection, prefer glucose 5% solution or saline buffer (HBS, PBS, normal saline, Ringer's solution).
8. Do not inject more than 1 mL of *In vivo* ViroMag per animal.
9. Do not inject complexes if precipitate has formed
10. Do not freeze magnetic nanoparticles
11. Do not add anything to the stock solution of magnetic nanoparticles
12. Magnet manipulation:
    a. Manipulate carefully the magnets. Danger of injury by strong magnetic attraction of ferromagnetic material
    b. Keep away from electronic devices and magnetic storage devices
    c. Persons with cardiac pacemakers should not work with these magnets

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

www.ozbiosciences.com

Any questions?

tech@ozbiosciences.com

In vivo ViroMag Reagent | Specifications

Package content	IV-VM30250: 250µL of *In vivo* ViroMag IV-VM30500: 500µL of *In vivo* ViroMag IV-KC30230: 250µL of *In vivo* ViroMag + a Magnets set (IV-MAG1) IV-TK30230: 50µL of *In vivo* ViroMag + 1 cylinder magnet (ø 10mm) IV-MAG1, Magnet Set: 1 extra small cylinder (ø 2mm), 1 small cylinder (ø 5mm), 1 cylinder (ø 10mm), 1 square (18x18 mm) magnets IV-MAG2, Square Magnets set: 4 square magnets (18x18 mm) IV-MAG3, Cylinder Magnet set: 4 extra small cylinder (ø 2 mm), 4 small cylinder (ø 5 mm), 4 cylinder (ø 10 mm) magnets
Shipping conditions	Room Temperature
Storage condition	Store the *In vivo* ViroMag reagent at +4°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled
Product Description	*In vivo* ViroMag is an optimized nanoparticles formulation dedicated to viral vectors for *in vivo* transduction. It is particularly suitable for Lentiviral/Retroviral, Adenoviral and Adeno-Associated Viral (AAV) vectors.
Important notice	For research use only. Not for use in diagnostic procedures

Applications

1. Viral Vectors

In vivo ViroMag has been developed for *in vivo* targeted transduction with any viral vectors. Virus/nanoparticles can be easily administrated through various injection routes such as systemic administration (intravenous, intra-artery) or local administration (intraperitoneal, intratumoral, intracerebroventricular, intramuscular).

Optimal conditions may vary depending on the viral vector, animal model, administration route and the target organ. Therefore, use the Table 1 as a starting point for volume of injection in mouse and rats and Table 3 for viral vectors amount.

Mouse		
Route of injection	Total volume of injection according to animal weight	Site of injection
Intravenous	200 µL (10-25 µL/g)	Tail vein
Intramuscular	100 µL (50 µL x 2 sites of injection)	Caudal tigh
Subcutaneous	200 µL (10-40 µL/g)	Scruff
Intraperitoneal	400 µL (20 µL/g)	Lower Ventral Quadrants
Intratumoral	100 µL (0.5 µL/mm^3)	Tumor
Intracerebroventricular	2 µL	Brain ventricle
Rat		
Route of injection	Total volume of injection	Site of injection
Intravenous	2.5 mL (10-20 µL/g)	Tail vein, saphenous vein
Intramuscular	300 µL (100 µL x 3 sites of injection)	Triceps, Quadriceps, Gluteals
Subcutaneous	1.25 mL (5-10 µL/g)	Scruff, Back, Abdomen
Intraperitoneal	2.5 mL (10-20 µL/g)	Lower Ventral Quadrants
Intratumoral	100 µL (0.5 µL/mm^3)	Tumor
Intracerebroventricular	10 µL	Brain ventricle

Table 1: Suggested volume of injection in mouse (20 g) and rat (250 g)

For more detailed protocols, see our Applications Notes on our website www.ozbiosciences.com or contact us at tech@ozbiosciences.com.

2. Magnets

Several kinds of magnets are provided with the *In vivo* ViroMag kit; use Table 2 to choose the best one adapted to your application.

Kind of magnet	Tissue
Extra Small Cylinder 2 mm (diameter) x 5 mm (height)	• Brain area • Endothelial cells • Small tumors • Lymph node • Ovary • Adrenal gland
Small Cylinder 5 mm (diameter) x 5 mm (height)	• Subcutaneous tumors • Salivary gland • Brain
Cylinder 10 mm x 5 mm (height)	• Subcutaneous tumors • Pancreas • Spleen
Square 17 mm (length) x 17 mm (length) x 5mm (height)	• Large organs • Large tumor • Muscle • Lung • Skin flap

Table 2: Examples of use of magnets

OZ Biosciences is currently proposing only those magnets. If you need specific magnet in terms of shape and size, please contact our technical service for obtaining fundamental properties of the magnet to purchase.

Protocol

Determine the required injection volume according to the Table 1. The amount of virus you need to inject is closely correlated to your viral preparation, the route of injection, the target tissue and your preliminary *in vitro* studies. Table 3 indicates a starting point for your protocol optimization. The *In vivo* ViroMag and injection solution should be at room temperature. You may use 10 to 20 µL of *In vivo* ViroMag per $1x10^6$ infectious viral particles. Do not exceed recommended volumes for injection.

Virus type	Viral particles titer (pfu)	*In vivo* ViroMag quantity (µL)
Adenovirus	$1x10^6$ to $5x10^8$	10-20
AAV	$1x10^6$ to $1x10^{10}$	10-20
Lentivirus	$1x10^6$ to $5x10^8$	10-20

Table 3: Suggested quantity of viral particles and *In vivo* ViroMag injected per mouse

1. Reagent Preparation

Immediately before use, vortex *In vivo* ViroMag vial. Add suitable amount of *In vivo* ViroMag to a sterile microtube (Table 3).

2. Complexes formation.

a. Add your virus preparation to the tube containing the *In vivo* ViroMag reagent and mix immediately by pipetting up & down.

b. Incubate 15-30 min at room temperature.

3. Injection.

a. Place the magnet on your targeted tissue

b. Slowly inject the complexes

c. Let the magnet stand from 5 min to 1 h (see table 4 and next section).
Notes for intracerebroventricular or intra tumoral injections: Place the magnet few seconds after the complexes injection. Dye *e.g.* Fast Green FCF can be added to the complexes solution for a better monitoring of the injection.

d. Monitor gene expression at the appropriate time point.

4. Magnetic incubation

The magnetic incubation time depends on the animal and the targeted tissue:

- for tumor, from 20 min (mouse, rat) to 1 hour (hamster, cat)
- for endothelial cells, from 5 to 20 min for mouse and rat, from 20 min to 1 h for rabbit or pig
- for peripheral tissue (*e.g.* stomach, gut, heart), 20 min
- for intracerebroventricular injection, 5 min

Refer to table 4, for other magnetic incubation times depending on target tissue, route of injection and magnet type.

Target tissue	Route of injection	Kind of magnet	Magnetic incubation
Tumor	Intravenous, Intra-arterial, Intratumoral	All kind	20 min to 1 h
Endothelial cells	Intravenous, Intra-arterial	Extra small Cylinder	5 min to 1 h
Heart	Intra-arterial	Cylinder	20 min
Liver	Intravenous	Cylinder, Square	10 min
Lung	Intravenous	Square	10 min
Pancreas	Intrapancreatic	Cylinder	20 min
Kidney	Intraperitoneal	Cylinder, Square	20 min
Gut	Ilea lumen	All kind	20 min
Stomach	Stomach lumen	Cylinder, Square	20 min
Brain	Intraventricular	Small Cylinder	5 min

Table 4: Suggested magnetic incubation time for various tissue

IMPORTANT NOTES:

- For long incubation time, (*e.g.* intratumoral injection), the magnet could be attached to the animal using adhesive tape in order to create a strong magnetic field in the area of the injection.
- Magnets can be easily handled with any magnetic surgical instruments (forceps, clamps, needle holders).
- Magnets can be sterilized by heat (steam sterilization or dry heat sterilization) or chemical means (ethanol 70%).

5. Bibliographic references

Please refer to the results sheet and to our website for a more exhaustive list of bibliographic references.

- Gupta A.K and Gupta M 2005 Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. *Biomaterials.* 26:3995-4021.
- Laurent N, Sapet C, Le Gourrierec L, Bertosio E and Zelphati O 2011 Nucleic acid delivery nanoparticles: the Magnetofection™ technology. *Therapeutic Delivery*. 2:471:482.
- Plank C, Zelphati O and Mykhaylyk O. 2011 Magnetically enhanced nucleic acid delivery. Ten years of magnetofection-progress and prospects. *Adv Drug Deliv Rev*. 63:1300-1331

Related products for *in vivo* applications

- **BrainFectIN** enables nucleic acids delivery into central nervous system of small animals.
- ***In vivo* DogtorMag** a cationic lipid-based magnetic nanoparticles formulation, designed for *in vivo* targeted transfection of nucleic acids.
- ***In vivo* SilenceMag** a cationic polymer-based magnetic nanoparticles formulation, designed to transfect small RNA, into target cell/tissue in vivo.

Purchaser Notification

Limited License
The purchase of the In vivo ViroMag grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed this protocol). This reagent is intended for in-house research only by the buyer. Such use is limited to the 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. Separate licenses are available from OZ Biosciences for the express purpose of non-research use or applications of the In vivo ViroMag. 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 In vivo ViroMag reagents and documentation to OZ Biosciences, or by destroying all in vivo ViroMag components. Purchasers are advised to contact OZ Biosciences with the notification that a In vivo ViroMag 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 In vivo ViroMag 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
In vivo ViroMag 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

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