Transfection reagent

In Vivo SilenceMag

In Vivo Nucleic Acids delivery

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. It is suggested to use 1 µL of *In vivo* SilenceMag per µg of RNA in initial experiments.
3. Allow reagents to reach RT and gently vortex them before forming complexes.
4. The final RNA 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* SilenceMag 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?

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In vivo SilenceMag Reagent | Specifications

Package content	IV-SM30500: 500µL of *In vivo* SilenceMag IV-SM31000: 1mL of *In vivo* SilenceMag IV-KC30240: 500µL of *In vivo* SilenceMag + a Magnets set (IV-MAG1) IV-TK30240: 100µL of *In vivo* SilenceMag + 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 condition	Room Temperature
Storage conditions	Store the *In vivo* SilenceMag transfection reagent at +4°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled
Product Description	*In vivo* SilenceMag is a cationic lipid-based magnetic nanoparticles formulation, designed for in vivo delivery of small RNA (siRNA, miRNA…).
Important notice	For research use only. Not for use in diagnostic procedures

Applications

1. Nucleic acids

In vivo SilenceMag has been developed for *in vivo* targeted transfection of small RNA (*siRNA, miRNA*). RNA/nanoparticles can be easily administrated through various injection routes such as systemic administration (intravenous, intra-artery) or local administration (intraperitoneal, intratumoral, intracerebroventricular, intramuscular).

The instructions given hereunder represent protocols that were successfully applied in several studies. Nevertheless, optimal conditions may vary depending on the nucleic acid, the animal model, the administration route and the target organ. Therefore, use the Table 1 as a starting point for RNA amount and volume of injection in mouse.

Route of injection	*Amount of nucleic acid per injection*	*Amount of nucleic acid per body weight*	*Total volume of injection according to animal weight*	*Site of injection*
Intravenous	*10 to 100 µg*	*0.5 to 5 mg/kg*	*200 µL (10-25 µL/g)*	*Tail vein*
Intramuscular	*10 to 40 µg*	*0.5 to 2 mg/kg*	*100 µL (50 µL x 2 sites of injection)*	*Caudal thigh*
Subcutaneous	*10 to 100 µg*	*0.5 to 5 mg/kg*	*200 µL (10-40 µL/g)*	*Scruff*
Intraperitoneal	*5 to 200 µg*	*0.2 to 10 mg/kg*	*400 µL (20 µL/g)*	*Lower Ventral Quadrants*
Intratumoral	*2 µg to 50 µg*	*0.1 to 2.5 mg/kg*	*100 µL (0.5 µL/mm^3)*	*Tumor*
Intracerebroventricular	*0.1 to 0.5µg/injection*		*2 µL*	*Brain ventricle*

Table 1: Suggested amount of nucleic acid and volume of injection in mouse

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* SilenceMag 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

Please refer to Table 1 to determine the required amount of DNA as well as volume injection. The DNA, *In vivo* Dogtor, *in vivo* CombiMag and injection solution should be at room temperature. We recommend using 1 µL of *In vivo* Dogtor and 1µL of *In vivo* CombiMag per µg of DNA.

Please refer to Table 1 to determine the required amount of siRNA/miRNA as well as injection volume. The nucleic acid, *In vivo* SilenceMag and injection solution should be at room temperature. We recommend using 1 µL of *In vivo* SilenceMag per µg of siRNA/miRNA.

1. Reagent Preparation

a. *In vivo SilenceMag*. Before each use, vortex *In vivo* SilenceMag vial. Add the required volume of *In vivo SilenceMag* (according to small RNA amount needed) to a sterile microtube.

b. *siRNA/miRNA solution*. Dilute small RNA in the final injection volume in a sterile vial (subtract the *In vivo* SilenceMag volume).

2. Complexes formation.

a. Add the small RNA solution to the *In vivo* SilenceMag and mix immediately by pipetting up and down.

b. Incubate the complexes for 20 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 (Table 3 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

See Table 3, 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 3: 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.

- 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
- Chen J, Zhu S, Tong L, Li J, Chen F, Han Y, Zhao M and Xiong W. 2014 Superparamagnetic iron oxide nanoparticles mediated ^{131}I-hVEGF siRNA inhibits hepatocellular carcinoma tumor growth in nude mice. *BMC Cancer* 14:114
- Fujiu K, Shibata M, Nakayama Y, Ogata F, Matsumoto S, Noshita K, Iwami S, Nakae S, Komuro I, Nagai R, Manabe I. 2017 A heart-brain-kidney network controls adaptation to cardiac stress through tissue macrophage activation. *Nat Med*. 23:611-622.

Related products for *in vivo* applications

- **BrainFectIN** enables nucleic acids delivery into central nervous system of small animals.
- ***In vivo* PolyMag** a cationic polymer-based magnetic nanoparticles formulation, designed for *in vivo* targeted transfection of nucleic acids.
- ***In vivo* ViroMag** an optimized nanoparticles formulation dedicated for *in vivo* transduction.

Purchaser Notification

Limited License

The purchase of the In vivo SilenceMag 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 SilenceMag. 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 SilenceMag reagents and documentation to OZ Biosciences, or by destroying all in vivo SilenceMag components. Purchasers are advised to contact OZ Biosciences with the notification that a In vivo SilenceMag 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 SilenceMag 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 SilenceMag 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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Rev 10/2020 NM