We Bring The World Of Biotechnology To You

BOCA SCIENTIFIC

Transfection reagent

SilenceMag™

siRNA Delivery Reagent

Protocol

Magnetofection Technology

This reagent needs to be used with a magnetic plate

OZBIOSCIENCES

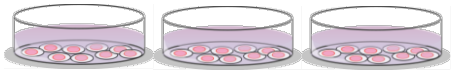
The art of delivery systems

SilenceMag™ Quick Protocol

To find your ideal silencing conditions with SilenceMag™, we suggest to test increasing doses of siRNA (or miRNA): from 10 to 50nM per well.

1

Seed cells to be at 50-70% confluent the day of transfection*



2

Prepare 3 tubes of siRNA (with different amounts of nucleic acids)*

96 well plate	24 well plate	6 well plate
10 nM/25nM/50nM in 50µL serum-free medium or buffer*	10 nM/25nM/50nM in 100µL serum-free medium or buffer*	10 nM/25nM/50nM in 200µL serum-free medium or buffer*

3

Prepare 3 tubes of SilenceMag™ (with different amounts of reagent)*

96 well plate	24 well plate	6 well plate
0.5µL/1µL/1µL in an empty microtube	1µL/2µL/3µL in an empty microtube	4µL/8µL/10µL in an empty microtube

4

Mix each tube of siRNA (step 2) to each tube of SilenceMag™ (step 3)

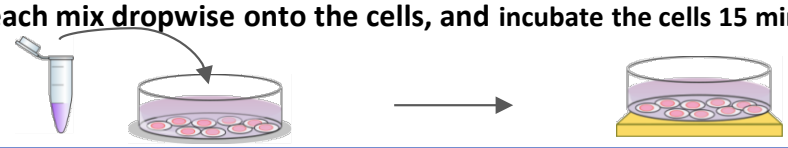
96 well plate			24 well plate			6 well plate		
siRNA		*SilenceMag*	*siRNA*		*SilenceMag*	*siRNA*		*SilenceMag*
10nM	+	0.5µL	10nM	+	1µL	10nM	+	4µL
25nM	+	1µL	25nM	+	2µL	25nM	+	8µL
50nM	+	1µL	50nM	+	3µL	50nM	+	10µL

5

Incubate 20 min at room temperature

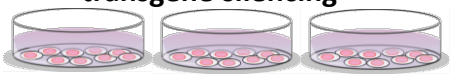
6

Distribute each mix dropwise onto the cells, and incubate the cells 15 min on the magnetic plate



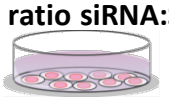
7

Remove the cells from the magnetic plate and incubate cells for 24 to 72h at 37°C until evaluation of transgene silencing



8

Choose the best ratio siRNA:SilenceMag™



These conditions might require some further optimizations depending on your cells, siRNA, target, etc.

* Please refer to the following section "Important Notes"

IMPORTANT NOTES – Before you begin

- ✓ The siRNA optimal concentration required to achieve the best gene silencing effect depends highly on the cells, target and siRNA sequence; consequently, we suggest to first test a range of siRNA concentration from 10 to 50nM.

- ✓ For cell lines, seed the cells 24h before transfection in a 96-well plate, 24-well plate or 6-well plate in respectively 150 µL, 400 µL and 2 mL of complete culture medium.

- ✓ Allow reagents to reach RT and gently vortex them before forming complexes.

- ✓ **Medium or buffer without serum & supplement** must be used for the siRNA/SilenceMag complexes preparation. Culture medium such as DMEM or OptiMEM or buffers such as HBS or PBS are recommended. In contrast, we do not recommend RPMI for preparing the complexes.

- ✓ We recommend respecting the order of addition of reagents: add the siRNA solution into the SilenceMag tube.

- ✓ Dilute the reagent with deionized water for doses less than 1µL.

- ✓ For most cell types, a medium change is not required after Magnetofection. However, it may be necessary for cells that are sensitive to serum/supplement concentration. This can be done immediately after the 20min incubation on the magnetic plate while keeping the cells onto the magnetic device, or 4 to 6h post-Magnetofection. Alternatively, the cells may be kept in serum-free medium during Magnetofection (up to 4 h). In this case, a medium change will be required after Magnetofection.

SilenceMag Reagent | Specifications

Package content	SM10200: 200 µL of SilenceMag reagent SM10500: 500 µL of SilenceMag reagent SM11000: 1 mL of SilenceMag reagent SM13000: 3 x 1 mL of SilenceMag reagent KC30300: 200 µL of SilenceMag reagent + Super Magnetic Plate KC30400: 200 µL of SilenceMag reagent + 100µL of PolyMag Neo reagent + 100µL PolyMag reagent + 100µL of CombiMag reagent + Super Magnetic Plate
Shipping conditions	Room Temperature
Storage conditions	Store the SilenceMag transfection reagent at +4°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled
Product Descriptions	SilenceMag is a magnetic nanoparticles formulation specifically designed for siRNA transfection. SilenceMag gives reliable high protein knockdown at very low doses of siRNA in numerous cell types (primary cells, hard-to-transfect & cell lines).
Important notice	For research use only. Not for use in diagnostic procedures

Protocol | siRNA in adherent cells

1. Cell preparation

It is recommended to seed or plate the cells the day prior transfection, however cells can also be prepared few hours before the transfection. Suspension cells should be prepared in the adequate vessel just before the transfection. The suitable cell density will depend on the growth rate and the conditions of the cells. Best results are achieved if cells are at least 50-70 % confluent at the time of Magnetofection (see the suggested cell number in the table below).

Culture vessel	Number of adherent cells	Number of suspension cells	Cell overlay volume
96-well	6 – 12 x 10^3	4 – 8 x 10^4	100 µL
24-well	4 – 8 x 10^4	25 - 50 x 10^4	400 µL
6-well	2 – 4 x 10^5	1 – 2 x 10^6	1800 µL

Table 1: Recommended number of cells to seed

2. siRNA/SilenceMag complexes preparation

a. *siRNA solution*. Dilute the siRNA stock solution (for instance 1µM stock solution) in 50 or 100 µL (refer to table 2) of culture medium without serum and antibiotics.

Culture vessel	96-well		24-well		6-well	
Dilution serum-free medium	50µL		50 µL		100 µL	
*Amount of siRNA (1 µM stock)**						
Final siRNA concentration	(µL)	(ng)	(µL)	(ng)	(µL)	(ng)
10 nM	2	27	5	67.5	20	270
20nM	4	54	10	135	40	540
50 nM	10	135	25	337.5	100	1350

* ng of siRNA was calculated on the basis of a MW = 13 500

Table 2: Suggested dilution procedure and amount of siRNA to test

b. *SilenceMag preparation*.
   i. SilenceMag solution should have an ambient temperature and be gently vortexed prior to use.
   ii. Add 0.5 to 10 µL of SilenceMag in an empty microtube (refer to table 3).

Culture vessel	96-well	24-well	6-well
Dilution serum-free medium	50 µL	50 µL	100 µL
Final transfection Volume	200 µL	500 µL	2 mL
Final siRNA concentration	*Amount of SilenceMag (µl)*		
10 nM	0.5	1	4
20nM	1	2	8
≥ 50 nM	1	3	10

Table 3: Recommended amount of SilenceMag per nM of siRNA used

c. Add siRNA solution to the SilenceMag tube and mix immediately 4-5 times by vigorous pipetting.

d. Incubate the mixture for 15-20 min at room temperature. Do not vortex or centrifuge!

3. Transfection

a. Add the complexes onto the cells and homogenize by gently rocking the plate side to side to ensure a uniform distribution of the mixture.

b. Place the cell upon the magnetic plate for 15 min.

c. Remove the plate and cultivate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of gene knockdown analysis.

NOTE: Depending on the siRNA amount, the gene targeted and the cell type, assays can be monitored 24 to 96h post-transfection.

IMPORTANT OBSERVATIONS

- Cell culture conditions: Best results are achieved when cells are 50–70 % confluent at the time of the transfection. If necessary, you can wash the culture medium containing the transfection mixture after 8-24h and replace it by fresh medium.
- siRNA concentration: We often observed good siRNA effects at very low concentrations from 0.1 to 5 nM. However, the efficiency may depend on the cell line, the target (half-life, expression level…) and the siRNA used. Consequently, we suggest you to start by testing a range of siRNA concentrations in order to obtain the best experimental conditions.
- Saving materials: In order to use less siRNA during your experiments you can also reduce the total transfection volume for the first 24h and then add more, fresh complete medium to maintain the cells in good conditions.
- Time course: The gene silencing time course depends on the amount/concentration of siRNA used. Indeed, with high quantity of siRNA, very efficient gene expression knockdown can be observed at earliest time point such as 16 or 24h. In contrast, with low siRNA concentration gene silencing require longer incubation such as 48 or 72h.

Protocol | siRNA in suspension cells

1. Cell preparation

The day before transfection split the cells at a density of 2 to 5 x 10^5 cells / mL, so they are in excellent condition on the day of transfection. Incubate overnight in complete culture medium.

2. siRNA/SilenceMag complexes preparation

The siRNA and SilenceMag solutions should have an ambient temperature and be gently vortexed prior to use.

a. *siRNA solution*. Dilute the siRNA stock solution (for instance 1µM stock solution) in 50 or 100 µL (refer to table 2) of culture medium without serum and antibiotics.

b. *SilenceMag preparation*.

i. SilenceMag solution should have an ambient temperature and be gently vortexed prior to use

ii. Add 0.5 to 10 µL of SilenceMag in an empty microtube (refer to table 3).

c. Add siRNA solution to the SilenceMag tube and mix immediately 4-5 times by vigorous pipetting.

d. Incubate the mixture for 15-20 min at room temperature. Do not vortex or centrifuge!

3. Transfection

a. While SilenceMag and siRNA incubate, dilute the cells in serum containing medium (with or without serum- or supplement; depending on the cell type and sensitivity of cells towards serum-free conditions) as suggested in Table 1 and perform one of the following three options to sediment the cells at the bottom of the culture dish in order to promote the contact with the magnetic nanoparticles:

i. Seed the cells on polylysine-coated plates and use the protocol for adherent cells

OR

ii. Briefly, centrifuge the cells (2 min) to pellet them and use the protocol for adherent cells.

OR

iii. Mix cell suspension with 20-30 µL of *CombiMag* (Magnetofection) reagent per 1 ml of cell suspension and incubate for 10 - 15 min. Then, distribute the cells to your tissue culture dish placed upon the magnetic plate and incubate for 15 more minutes.

b. Add the SilenceMag / siRNA mixture to the cells (while keeping the cell culture plate on the magnetic plate if you proceeded using the method described just above).

c. Incubate for 15 min.

d. Remove culture plate from magnetic plate.

Optimization Protocol

In order to get the best out of SilenceMag™, several parameters can be optimized:

- siRNA dose used, which strongly depends on the efficiency and specificity of your siRNA
- Ratio of SilenceMag to siRNA
- Cell type and cell density
- Incubation time

1. Start by optimizing the siRNA dose with the fixed ratio of SilenceMag /siRNA that has been previously optimized.

2. Thereafter, change the ratio SilenceMag / siRNA. To this end, use a fixed amount of siRNA and vary the amount of SilenceMag from 2 times less up to three times more than the suggested amount detailed in the Table 3. For instance, from 0.5 to 3µL of SilenceMag in a 24-well plate or from 2 to 12µL of SilenceMag in a 6-well plate for 10 nM siRNA. The ratio of SilenceMag / siRNA can be changed by doubling or multiplying the volumes of the reagents used. Similarly, the reagents can be pre-diluted in deionized water and aliquots of the resulting dilutions are incubated with siRNA.

3. After having identified the correct quantity of SilenceMag and siRNA, you could pursue the process by optimizing the cell number (density) and time course of your experiment.

Additional products for your silencing experiments

- **Lullaby** for siRNA transfection
- **Lullaby Stem** for siRNA transfection into stem cells

Purchaser Notification

Limited License

The purchase of this product 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). This reagent is intended for in-house research only by the buyer. Such use is limited to the purposes 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 this product. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, please contact us. Buyers may end this License at any time by returning the product and documentation to OZ Biosciences, or by destroying all components. Purchasers are advised to contact us with the notification that the product 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 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.

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Rev. 02/2018