

RNAi Mate



RNAi-Mate transfection reagents can be used in vivo and in vitro for nucleic acid including DNA, RNA, antisense oligo and siRNA, and also can be used in co-transfection for DNA/siRNA.

RNAi is a very popular research technology in biology and biomedical research. The commercially available siRNA transfection reagents can not meet the needs for high-throughput siRNA transfection experiments. Therefore, transfection reagents Cation-liposome "RNAi-Mate" was developed, which is highly efficient compared to the market-leading reagent.

Applications:

- * Transfection of primary culture and transformation of cell strain gene
- * High-throughput transfection of siRNA
- * DNA transfection; Co-transfection of DNA and siRNA
- * In vivo delivery of siRNA oligos (siRNA, DNA and RNA)
- * Transfection of adherent cell and suspension cells

Features:

- *No necessary to change culture media. Easy to operate. Good repeatability.
- *Transfect siRNA oligos in high efficiency
- *High transfection efficiency can be obtained even in culture media containing serum.
- *Can be shipped at room temperature. Can be stored at 4 °C for long time.
- *No cell toxicity.

Cat.NO. : SI-MATE-1000

Price : 285 euros

Quantity : 1.0mL (83 times/35mm, 1mg/mL)

Cat.NO. : SI-MATE-100

Price : 35 euros

Quantity : 100 µL (8 times/35mm, 1mg/mL)

Procedure

Transfection in vitro

1. Cell culture

RNAi-mate can be used for the transfection of DNA and siRNA into many different kind cells. RNAi Mate siRNA transfection reagents have been extensively tested in many different kinds of cell lines originated from different sources, ranging from standard lines, e.g. HeLa, MC-7, Hep3B, COS-7, Neuro-2a, NIKS, B16, DLD-1, NIH/3T3, HT-29, A549, CHO-K1 and 293, and SVRbag4. It is advised that before starting your transfection experiment, you put your cells on your cell plate, then add proper culture medium, lastly incubate cells for 24 hrs to be 40%-70% by confluence.

Table 1 cell culture vessel and operation

Cell culture plating format	Surface area (mm ² /well)	Cell density per well	Culture medium (μL /well)
96 well plate	50	1.5x10 ⁴ -5.0x10 ⁴	100μL
48 well plate	100	3.0x10 ⁴ -1.0x10 ⁵	200μL
24 well plate	200	8.0x10 ⁴ -2.0x10 ⁵	500μL
12 well plate	401	1.6x10 ⁵ -4.0x10 ⁵	1.0 mL
6 well plate	962	3.0x10 ⁵ -8.0x10 ⁵	2.0 mL
35 mm	962	3.0x10 ⁵ -8.0x10 ⁵	2.0 mL
60 mm	2827	1.0x10 ⁶ -2.5x10 ⁶	6.0 mL

2. Choose the appropriate ratio of RNAi-Mate: siRNA/DNA

The appropriate ratio of RNAi-Mate: siRNA/DNA is very crucial to achieve high efficiency for transfection. It is recommended that the appropriate ratio of RNAi-Mate :siRNA(DNA) is 1-4:1 (W:W) . You can get good results in the range.

Table 2 Recommended quantity of RNAi-Mate: DNA for DNA transfection

Cell culture plating format	DNA	Volume of the culture medium	RNAi-Mate
96 well plate	0.2μg	100μL	0.6μg
24 well plate	0.8μg	500μL	2.4μg
12 well plate	1.6μg	1 mL	4.8μg
6 well plate	4.0μg	2 mL	12μg
35 mm	4.0μg	2 mL	12μg
60 mm	8.0μg	5 mL	24μg

Table 3 Recommended quantity of RNAi-Mate: siRNA for siRNA transfection

Cell culture plating format	siRNA	Volume of the culture medium	RNAi-Mate
96 well plate	0.3µg	100µL	0.9µg
24 well plate	1µg	500µL	3µg
12 well plate	2µg	1 mL	6µg
6 well plate	5µg	2 mL	15µg
35 mm	5µg	2 mL	15µg
60 mm	10µg	5 mL	30µg

3. Adherent Cell transfection procedure:

This procedure is suited to adherent cell transfection using 24-well plate.

Choosing healthy cell is very important for enhancing transfection efficiency. The quantity of siRNA(DNA) and DNA and the ratio between the siRNA(DNA) and RNAi-Mate can be adjusted slightly within the recommended range.

3.1 One day before transfection, incubate $4-5 \times 10^4$ cells into 24-well plate; add 0.5mL culture medium containing FBS and antibiotics

3.2 Choose the appropriate cell quantity from primary incubation to make sure that cell fusion can reach 40-70%.

3.3 Dilute 1µg siRNA(or 0.8µg DNA) in 100µl serum-free medium, add 3µg RNAi-Mate reagent (when transfect DNA, 2.4µg RNAi-Mate reagent is added) mix thoroughly, incubate at room temperature for 30min in order to form siRNA/RNAi-Mate (or DNA/RNAi-Mate) complex.

3.4 Add siRNA/RNAi-Mate (or DNA/RNAi-Mate) complex into culture medium and mix up gently.

3.5 After incubating cells at 37°C for 24h-120h, continue other steps for transfection.

4. Suspension cells transfection procedure.

This procedure is suited to suspension cell transfection using 24-well plate.

Choosing healthy cell is very important for enhancing transfection efficiency. The quantity of siRNA(DNA) and DNA and the ratio between the two can be adjusted slightly within the recommended range.

4.1 On the day you start transfection, collect cells and centrifugate, then resuspend in the FBS culture medium.

4.2 Dilute 1µg siRNA(or 0.8µg DNA) in 100µl serum-free medium, add 3µg RNAi-Mate reagent (when transfect DNA, 2.4µg RNAi-Mate reagent is added) , then add the above mixture into the wells of the 24-well plate.

4.3 Incubate at room temperature for 30min in order to form siRNA/RNAi-Mate (or DNA/RNAi-Mate) complex.

4.4 Add 400µL cell suspension solution (cell quantity is determined by cell type and the time needed for analysis after transfection) .

4.5 After incubating cells at 37°C for 24h-120h, continue other steps for transfection.

5. DNA and siRNA co-transfection

5.1 One day before transfection, incubate $4-5 \times 10^4$ cell into 24-well plate, add 0.5mL culture medium containing FBS and antibiotics

5.2 Choose the appropriate cell quantity of primary incubation to make sure that cell fusion can reach 40-70%.

5.3 Dilute 1 μ g siRNA(or 0.8 μ g DNA) in 100 μ l serum-free medium, add 3 μ g RNAi-Mate reagent (when transfect DNA, 2.4 μ g RNAi-Mate reagent is added) mix thoroughly, incubate at room temperature for 30min in order to form siRNA/RNAi-Mate (or DNA/RNAi-Mate) complex.

5.4 Add siRNA/RNAi-Mate (or DNA/RNAi-Mate) complex into culture medium and mix up gently.

5.5 After incubate cells at 37°C for 24h-120h, continue other steps for transfection.

Transduction in vivo

This procedure is suited to experiments siRNA、DNA and siRNA/DNA transduction in vivo.

1. Appropriate amount siRNA and DNA is dissolved in RNAase-free sterile water, mix up gently; because volume of the injection is limited, we suggest high concentration siRNA or DNA, generally DNA is 2 μ g / μ L、siRNA is 10 μ g / μ L.

Mix up appropriate amount DNA、siRNA or siRNA/DNA complex with RNAi-Mate. For example, in No.1 tube add 0.5 μ L of DNA (1 μ g) and 0.5 μ L of siRNA (5 μ g), in No.2 tube add 0.55 μ L of RNAi-Mate (24 μ g) and 0.45 μ L of RNAase-free sterile water , then collect No.1 tube solution and add them into No.2 tube , incubate at room temperature for 30min , in order to form siRNA/RNAi-Mate (or DNA/RNAi-Mate) complex.

2. Preparation siRNA/DNA-RNAi-Mate complex can be used to transduct siRNA、DNA siRNA/DNA in vivo.

FAQs and Suggestion

1. Low transfection efficiency

Problem	Suggestion
Not optimized RNAi-Mate: siRNA(DNA) ratio	Optimize RNAi-Mate: siRNA(DNA), ratio is 1—4: 1 (W:W)
Concentration of siRNA/RNAi-Mate (or DNA/RNAi-Mate) complex is low	Slightly increase concentration of siRNA/RNAi-Mate (or DNA/RNAi-Mate) complex

Condition of cell growth is bad	Cells with non-optimal conditions decrease transfection efficiency. Suggest that cell fusion can reach 40-70% in 24hrs after incubation, finishing transfection operation in 24hrs.
Purify of DNA or siRNA is too low	Use high-purify DNA or siRNA, ideally use column purified DNA and HPP grade siRNA
The culture medium used to dilute the DNA or siRNA containing serum	Generally, serum can not depress the formation of siRNA/RNAi-Mate (or DNA/RNAi-Mate) complex dramatically, suggest using serum-free culture medium to dilute DNA or siRNA.

2. The repeatability is bad

Cell fusion is not uniform	Using the same amount of master cell, cultural time and cultural conditions after incubating must be uniform
Times of cell subculture is too many	Using low subculture times cells

3. Cells died apparently

Problem	Suggestion
Key gene related to cell survive is shut down	Re-design the experiment
Cell conditions is not very good	Using low subculture times cells and cell fusion can reach 40-70% in 24hrs after incubation, finishing transfection operation within 24hrs.
Concentration of siRNA/RNAi-Mate (or DNA/RNAi-Mate) complex is too high	Generally, siRNA/RNAi-Mate (or DNA/RNAi-Mate) complex would not affect cell growth, but when concentration is too high, sometimes it may produce some cell toxicity

4. Gene expression or gene silencing efficiency is lower

Problem	Suggestion
Expression vector design is not correct or siRNA design is not correct	Re-design the experiment

Cultural time is too short after transfection

Gene expression need certain time, so prolong the culture time appropriately in necessary.
