

# RNAi-Mate

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## 1. Introduction:

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. RNAi-Mate is highly efficient compared to the market-leading reagent.

## 2. 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

## 3. Features:

- \* Not 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.

## 4. Procedure:

### 4.1. Transfection in vitro

#### 4.1.1. Cell culture

RNAi-mate can be used for the transfection of DNA and siRNA into many different kind cells. Our new 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, 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 <sup>2</sup> /well)	Cell density per well	Culture medium (uL/well)
96 well plate	50	1.5x10 <sup>4</sup> - 5.0x10 <sup>4</sup>	100uL
48 well plate	100	3.0x10 <sup>4</sup> - 1.0x10 <sup>5</sup>	200uL
24 well plate	200	8.0x10 <sup>4</sup> - 2.0x10 <sup>5</sup>	500uL
12 well plate	401	1.6x10 <sup>5</sup> - 4.0x10 <sup>5</sup>	1.0mL
6 well plate	962	3.0x10 <sup>5</sup> - 8.0x10 <sup>5</sup>	2.0mL
35mm	962	3.0x10 <sup>5</sup> - 8.0x10 <sup>5</sup>	2.0mL
60mm	2827	1.0x10 <sup>6</sup> - 2.5x10 <sup>6</sup>	6.0mL

#### 4.1.2. 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 2:1 - 4:1

(W:W). Generally, 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	0.2ug	100uL	0.6ug
24 well plate	0.8ug	500uL	2.4ug
12 well plate	1.6ug	1mL	4.8ug
6 well plate	4.0ug	2mL	12ug
35mm	4.0ug	2mL	12ug
60mm	8.0ug	5mL	24ug

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	0.3ug	100uL	0.9ug
24 well plate	1ug	500uL	3ug
12 well plate	2ug	1mL	6ug
6 well plate	5ug	2mL	15ug
35mm	5ug	2mL	15ug
60mm	10ug	5mL	30ug

### 4.1.3. 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 the ratio between the siRNA (DNA) and RNAi-Mate can be adjusted slightly within the recommended range.

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

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

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

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

4.1.3.5. After incubating cells at  $37^{\circ}\text{C}$  for 24-120hrs, continue other steps for transfection.

#### 4.1.4. 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 the ratio between the two can be adjusted slightly within the recommended range.

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

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

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

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

4.1.4.5. After incubating cells at  $37^{\circ}\text{C}$  for 24-120hrs, continue other steps for transfection.

#### 4.1.5. DNA and siRNA co-transfecion

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

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

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

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

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

## 4.2. Transduction in vivo

This procedure is suited to experiments siRNA, DNA and siRNA\DNA transduction in vivo.

4.2.1. 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 2ug/uL, siRNA is 10ug/uL.

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

4.2.3. Prepared siRNA/DNA-RNAi-Mate complex can be used to transduct siRNA、DNA siRNA\DNA in vivo.

## 5. FAQs and Suggestion:

Table-4. Low transfection efficiency.

Problem	Suggestion
Not optimized RNAi-Mate:siRNA(DNA) ratio	Optimize RNAi-Mate:siRNA(DNA) ratio. 2:1 - 4:1 (W:W) recommended.
Concentration of siRNA/RNAi-Mate (or DNA/RNAi-Mate) complex is too 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-purity 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.

Table-5. The repeatability is bad.

Problem	Suggestion
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.

Table-6. 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.

Table-7. Gene expression or gene silencing efficiency is low.

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 if necessary.

## 6. Quality guarantee and Service:

MDBio strictly examine RNAi-Mate gene transfection reagent batch to batch, and do experiments to validate the reagents, to make sure product quality meet the highest standard. If the reagent have quality problem, which is not caused by operating incorrectly, we offer exchange of the goods free of charge. Please read this RNAi-Mate technical manuals carefully before use our products.