Sirnafectamine Transfection Reagent

User's Instruction

Description

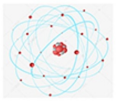
Sirnafectamine is a new generation of RNA transfection reagent, which can be used in RNA transfection experiment. Sirnafectamine accelerates the binding of glycoprotein on cell membrane, which promotes the endocytosis of cells. The unique feature of Sirnafectamine is that it can protect RNA during endocytosis, as well as when it stimulates endosome swelling and rupture. Sirnafectamine and RNA are released from endosomes at last. These features reduce the specific effect of non-target cells and enhance the high specific gene silencing. The transfection efficiency of Sirnafectamine is high. Very low concentration of siRNA can produce high gene silencing efficiency. Sirnafectamine can transfect a wide range of cell lines. In addition, compared with other siRNA transfection reagents, Sirnafectamine has minimal cytotoxicity and the best cell state after transfection. Sirnafectamine is also a ready-to-use siRNA transfection reagent, and the reagent is not affected by serum and antibiotics, so the operation is simple and fast.

Applications

- Transfection of primary culture cells and transformed cell lines.
- High throughput siRNA transfection test.
- DNA transfection.
- Co-transfection of DNA and siRNA.
- Transfection of adherent cells and suspension cells.

Features

- Ready-to-use
- No need to change the culture medium
- No RNase activity
- The reaction can be completed in half an hour
- Good repeatability

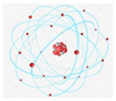


- High transfection efficiency for siRNA
- High transfection efficiency in serum containing medium
- Can be transported at room temperature and stored for a long time at 4°C
- Low cytotoxicity
- Widely used in cell culture
- Active in complete medium containing antibiotics

Protocol

When using Sirnafectamine to transfect RNA oligos into mammalian cells, the following general guidelines should be followed:

- To obtain the best gene knockdown results, the amount of siRNA or miRNA transfected into each cell line needs to be determined by experiments. If you are transfecting your cell line for the first time, it is recommended to try several concentrations of Sirnafectamine and change the concentration of siRNA or miRNA in the range of 1-100nM to determine the conditions required to achieve the optimal level of gene knockdown. High concentrations of siRNA or miRNA may be cell line dependent. We recommend starting with 5nM siRNA or miRNA.
- It is recommended to transfect at 30%-50% confluence. Generally, gene blocking analysis should be performed at least 24-72 hours after transfection. Low density transfection can minimize the cell activity damage caused by cell overgrowth. The extremely low cytotoxicity of Sirnafectamine can facilitate researchers to select suitable conditions for transfection according to the growth characteristics of target cells and the expression characteristics of target genes.
- The appropriate negative and positive controls are used to optimize the transfection and detection conditions. The negative control sequence labeled with fluorescence can be used to optimize the transfection conditions of cell lines. Once the optimal conditions for transfection are determined, it is recommended to include fluorescent labeled negative control sequences as an indicator of transfection efficiency in each experiment. For most cells, housekeeping gene is a good positive control. Transfect different concentrations of positive control siRNA into target cells (also suitable for experimental target siRNA), check the decreased levels of control protein or mRNA relative to untransfected after 48 hours. Excessive siRNA will lead to cytotoxicity and



even death.

- Avoid RNase contamination. Small amount of RNase will lead to the failure of siRNA/miRNA experiment. Because RNases are ubiquitous in the experimental environment, such as skin, hair, all objects that have been touched by hands or exposed to the air. Therefore, it is very important to ensure that each step of the experiment is not contaminated by RNase. Especially for the tips and tubes that directly contact with oligo stock solution, please ensure RNase free. We recommend to use commercial RNase-free tips and tubes.
- Healthy cell culture and strict operation are important for the repeatability of transfection. Generally, the transfection efficiency of healthy cells is higher. In addition, the stability of the cells used in each experiment can be ensured by the fewer generation. To optimize the experiment, it is recommended to use the transfected cells less than 50 passages, otherwise the transfection efficiency will decrease with time.

Materials

Prepare the following reagents before the experiment:

- Mammalian cell lines (before transfection, make sure that the cells are healthy and have a survival rate of more than 90%)
- siRNA or miRNA oligo (recommended to dissolve to 20μM)
- Sirnafectamine (stored at +4°C before use)
- Medium (preheat at 37°C before use)
- Suitable cell culture plate and others

Transfection steps (take 24 well plate as an example)

Follow these steps when using Sirnafectamine to transfect siRNA or miRNA oligo into mammalian cells. Refer to table 1-3 to determine the amount and volume of reagents added in different tissue culture. We recommend to use the final concentration of 5nM siRNA or miRNA oligo and the amount of Sirnafectamine recommended in Table 1 as the starting point for optimization. Customers shall optimize the target cell line and siRNA or miRNA oligo (the recommended final concentration range is 1-100nM).

According to the number of cells recommended in Table 1, inoculate the cells in 24 well plate a day before transfection, so that the confluence of cells is 30-50% at the time of transfection.

For each transfected sample, siRNA/miRNA oligo - Sirnafectamine complex is prepared

as follows:

1. Before transfection, dilute siRNA/miRNA oligo solution to 1μM (recommended storage concentration is 20μM, and is diluted to 1μM by adding 19μl RNase-free water). Add 3μl 1μm siRNA/miRNA oligo solution to 100μl Opti MEM or serum-free DMEM, serum-free 1640. Mix well and incubate at room temperature for 5 mins.

2. After incubation for 5 min, add 2μl Sirnafectamine into the diluted siRNA/miRNA oligo in 1). After addition, fully mix immediately (it can be vibrated with an oscillator or sucked with a pipette for more than 10 times). After slight centrifugation, place at room temperature for 10 min (please ensure enough time to incubate, and should not exceed 1 hour) to allow the formation of siRNA/miRNA oligo - Sirnafectamine complex.

3. Within 10 mins of incubation, the original medium should be removed from the cell culture plate. Add 500μl of complete medium (containing 10% serum and antibiotics) to each well. Sirnafectamine is not affected by serum and antibiotics.

4. Add siRNA/miRNA oligo - Sirnafectamine complex dropwise to each well containing cells and medium. Shake the plate back and forth gently and mix well. In Sirnafectamine transfection, there is no need to remove the complex or change the medium. If the target cells are sensitive to transfection (such as primary cells), we suggest that the complete medium can be changed 4-6 hours after transfection without loss of transfection activity.

5. Incubate at 37°C for 24-96 hours in CO_2 incubator until suitable for target gene analysis. We suggest that the target RNA can be collected in 24-48 hours for cell detection, and the target protein can be collected in 48-72 hours for detection.

Table 1. Recommended Cell Number and Culture Medium Volume

Culture Plate	Cell Number	Surface Area / Well (cm^2)	Volume / Well (ml)
96-well>	7 500 ± 2 500>	0.3>	0.2 ml>
48-well>	15 000 ± 5 000>	1>	0.5 ml>
24-well>	25 000 ± 10 000>	1.9>	1 ml>
12-well>	50 000 ± 20 000>	3.8>	2 ml>

6-well/ 35 mm>	150 000 ± 50 000>	9.4>	4 ml>
60 mm/ T25 flask>	400 000 ± 100 000>	28>	8 ml>
100 mm/ T75 flask>	1 000 000 ± 250 000>	78.5>	15 ml>

Table 2. Sirnafectamine Recommended Dosage (24 well plate)

siRNA/miRNA Final Concentration	Sirnafectamine Recommended Dosage
> 10 nM	3 ± 1 µl
≤10 nM	1 to 2 µl

Table 3. Recommended Dosage of Transfection in Different Culture Plate

Culture Plate	Surface Area to 24 Well Ratio	RNA (pmol)	Sirnafectamine Volume	RNA-Sirnafectamine Volume	Medium/ Well	Total Volume	RNA Final Concentration
96-well	0.2	0.85	1 ± 0.5 µl	50 µl	125 µl	175 µl	5nM
24-well	1	3	2 ± 1 µl	100 µl	500 µl	600 µl	5nM
12-well	2	6	4 ± 2 µl	200 µl	1 ml	1.2 ml	5nM
6-well/ 35 mm	5	11	8 ± 2 µl	200 µl	2ml	2.2 ml	5nM
60 mm/ T25 flask	10	22	15 ± 5 µl	400 µl	4ml	4.4 ml	5nM
100 mm/ T75 flask	15	52.5	40 ± 10 µl	500 µl	10ml	10.5 ml	5nM
96-well	0.2	0.85	1 ± 0.5 µl	50 µl	125 µl	175 µl	5nM
24-well	1	3	2 ± 1 µl	100 µl	500 µl	600 µl	5nM

We suggest to optimize the final RNA concentration according to your experiment.

Note

- The effective concentration of RNA and the quantity of transfection reagent need to be optimized. To get better results, the RNA quantity per well can be optimized from 1 nM to 100 nM.
- Different cell density may greatly affect the transfection efficiency and cytotoxicity. To obtain the best transfection effect, it is suggested to carry out different gradient transfection from 30% to 50% of cell density to determine the optimal transfection quantity.
- When preparing siRNA/miRNA oligo - Sirnafectamine complex, must use serum-free medium. OPTI-MEM, serum-free DMEM or serum-free 1640 are recommended.
- Generally speaking, the best time to observe the effect of RNAi at mRNA level is 24-48 hours after transfection, and the best time to observe the effect of RNAi at protein level is 48-72 hours after transfection.
- There is no special requirement for cell growth medium. Serum containing medium or antibiotics can be used.

Storage

The minimum shelf life is 2 years at 4°C.