LipoRibo Transfection Reagent

Product Number: TR0535

Shipping and Storage

Store at 4ºC, Long term non use can be stored at -20 º C.

Components

Component	TR0535	TR0535	TR0535
LipoRibo Transfection Reagent	0.5ml	1.5ml	5×1.5ml

Description

LipoRibo Transfer Agent is a newly developed, highly convenient and efficient small RNA based on nanomaterials, including siRNA MiRNA and other cell transfection reagents have achieved or even exceeded the transfection effect of international mainstream small RNA transfection reagents. Suitable for converting siRNA Transfection of miRNA or other forms of single or double stranded RNA or DNA small nucleic acids into eukaryotic cells can also be used for siRNA in living animals Transfection of small nucleic acids such as miRNA and its use in gene therapy.

Features

1. The nanotechnology of LipoRibo Transfer Agent ensures its reliability and stability during transient and stable transfection.
2. Convenient to use, serum-free culture medium, small RNA, and transfection reagents can be directly mixed and incubated at room temperature for 20 minutes before being directly added to the cell culture vessel, achieving convenient operation of cell transfection.
3. It has very high transfection efficiency, good reproducibility, simple operation, and extremely low cytotoxicity for common mammalian cells. It is suitable for both adherent cells and suspension cells, especially for adherent cells that are difficult to transfect.
4. Usually, there is no obvious cytotoxicity, so in most cases, there is no need to change the cell culture medium after transfection. In many cases, cells can be collected for targeted protein expression detection after about 48 hours of transfection. For some target proteins with longer half-lives, a significant decrease in protein levels can only be detected 72 to 96 hours after transfection with siRNA or miRNA.
5. LipoRibo Transfer Reagent has been tested on various cells, including mouse embryonic fibroblast NIH3T3, human embryonic kidney cell HEK293, cervical cancer cell Hela, and Chinese hamster ovary cell CHO, and the transfection efficiency can reach around 80-90%.
6. When transfecting cells, they are not affected by the serum and antibiotics in the culture medium, and can be transfected in the presence of serum and antibiotics.
7. The transfection effect can be quickly identified by transfecting fluorescent labeled small RNAs under a fluorescence microscope, or judged by combining Western detection results. The transfection effect of LipoRibo Transfer Agent on FITC labeled siRNA targeting STIM1 is shown in Figures 1 and 2.

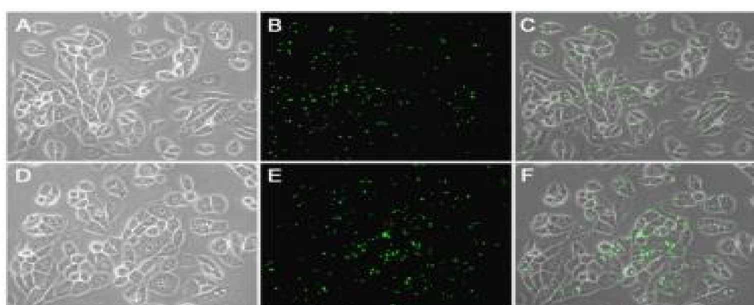

Figure 1. Real photos taken 4 hours after LipoRibo Transfer Reagent transfection of FITC labeled STIM1 siRNA into Hela cells (A-C) or NIH3T3 cells (D-F). FITC labeled STIM1 siRNAs adhere to the cell membrane or have entered the cell through endocytosis. A. B and C are the bright field images, fluorescence images, and their superimposed images of Hela cells, respectively. D. E and F are the bright field images, fluorescence images, and their superimposed images of NIH3T3 cells, respectively. Both NIH3T3 cells and Hela cells were seeded on a six well plate, and a mixture of 100pmol FITC labeled STIM1 siRNA and 4ul LipoRibo Transfer Agent was added to each well during transfection.

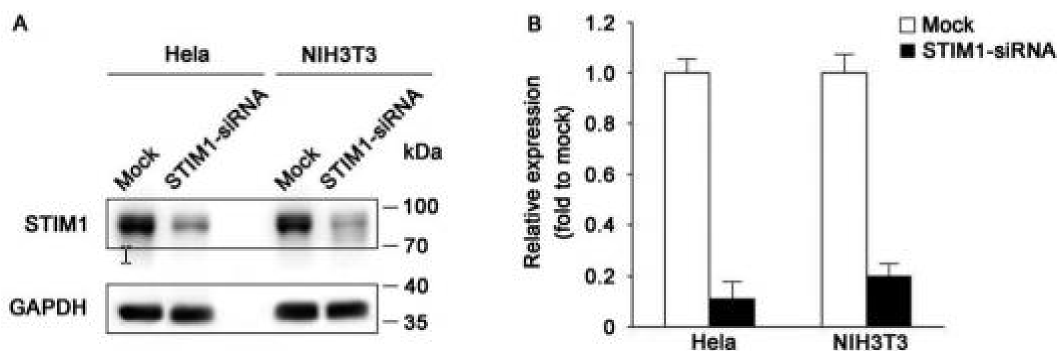

Figure 2. LipoRibo Transfer Reagent transfected FITC labeled STIM1 siRNA into Hela or NIH3T3 cells for 48 hours, and Western blot was used to detect the downregulation effect of STIM1 protein. The STIM1 antibody (AF2614 Stromal interaction molecule 1 Rabbit Monoclonal Antibody) used in Western blot was diluted at 1:1000 according to the instructions, and the secondary antibody horseradish peroxidase labeled goat anti rabbit IgG (H+L) (A0208) was diluted at 1:1000. GAPDH is an internal reference. A is the detection result of Western blot; B is the grayscale quantitative statistical map of Figure A and repeated experiments.

Each milliliter of this transfection reagent can transfect approximately 40 10cm culture plates, 125 6cm culture plates, 250 wells on 6-well plates, 625 wells on 12 well plates, 1250 wells on 24 well plates, 2500 wells on 48 well plates, and 6250 wells on 96 well plates.

Note

1. Increasing the amount of LipoRibo Transfer Reagent can easily lead to a decrease in transfection efficiency. Please prioritize transfection according to the recommended amount of transfection reagents. Adjust the dosage if necessary.
2. The use of high-purity small RNA and other small nucleic acids helps to achieve higher transfection efficiency.
3. Cells must be in a good growth state before transfection.
4. After testing, changing the cell culture medium 4-6 hours after transfection with this product did not have a significant impact on transfection efficiency. If necessary, the cell culture medium can be changed 4-6 hours after transfection.
5. Self provided serum-free culture medium without antibiotics Opti MEM ® culture medium or regular DMEM culture medium.
6. LipoRibo Transfer Agent should not be vortexed or centrifuged, and should be slowly shaken and mixed.
7. After using LipoRibo Transfer Agent, please immediately cover it to avoid prolonged exposure to the air, which may affect the transfection efficiency.
8. This product is only for scientific research purposes by professionals and cannot be used for clinical diagnosis or treatment, food or medicine, or stored in ordinary residential areas.
9. For your safety and health, please wear laboratory clothes and disposable gloves when operating.

Protocol

SiRNA transfection:

1. Cell culture (using a six well plate as an example, other culture plates or dishes can refer to the six well plate): On the day before transfection (18-24 hours), approximately 200000 to 700000 cells per well (the specific number of cells depends on cell type, size, and growth rate) are inoculated into the six well plate for cultivation, so that the cell density can reach about 70-80% the next day.

2. Before performing the following transfection steps, replace each well of the six well plate containing cells with 2ml of fresh culture medium (complete culture medium containing serum and antibiotics). For LipoRibo Transfer Reagent, the presence of antibiotics does not affect transfection efficiency, nor does it cause cytotoxicity after cell transfection.
3. Referring to the table below, take a clean and sterile centrifuge tube. For each well in the six well plate to be transfected, add 125ul of DMEM culture medium (high sugar DMEM or low sugar DMEM can be used) or Opti-MEM®Medium without antibiotics or serum. Add 100pmol of siRNA and gently blow with a gun to mix well; Add 4ul LipoRibo Transfer Agent and gently blow with a gun to mix well. Please be careful not to vortex or centrifuge. After preparation, stable at room temperature for 6 hours

	96-well	48-well	24-well	12-well	6-well	6cm dish	10cm dish
Serum free culture medium or Opti-MEM®Medium	5μl	12.5μl	25μl	50μl	125μl	250μl	750μl
siRNA	4pmol	10pmol	20pmol	40pmol	100pmol	200pmol	600pmol
LipoRibo Transfection Reagent	0.16μl	0.4μl	0.8μl	1.6μl	4μl	8μl	24μl
After adding siRNA, gently mix well. After adding LipoRibo Transfer Agent, gently mix well and incubate at room temperature for 20 minutes (refer to Figure 3, the time should not be less than 20 minutes).							
The amount of mixture added per well	5μl	12.5μl	25μl	50μl	125μl	250μl	750μl
Add a mixture of LipoRibo Transfer Agent and siRNA evenly to each well according to the above dosage, and continue cultivation directly without changing the culture medium after a few hours							

Note 1): For cells with one well in a six well plate, The dosage of LipoRibo Transfer Agent can be appropriately adjusted within the range of 2-6ul (if cytotoxicity is found, the dosage of LipoRibo Transfer Agent can be appropriately adjusted within the range of 2-4ul), The amount of siRNA can be appropriately adjusted within the range of 50-250 pmol. The usual ratio of siRNA dosage (pmol) to LipoRibo Transfer Reagent (ul) is 25:1. If necessary, the transfection effect can be optimized within the range of 10:1-40:1. The recommended ratio in the above table is 25:1. At this time, the dosage of LipoRibo Transfer Reagent is relatively small, which is both economical and efficient. The optimal transfection conditions vary depending on the cell type and culture conditions, and can be optimized within the recommended range.

2) The recommended concentration for siRNA is 20uM, with a commonly used concentration range of 10-50uM.

3) For the situation where multiple wells are transfected with the same amount of small RNA, the required amount of LipoRibo Transfer Agent and siRNA for each well can be increased by corresponding multiples, and then mixed together in the same centrifuge tube. After mixing, the recommended amount can be added dropwise to the cell culture vessel.

4) For other culture plates or culture vessels, the amount of various reagents can be converted proportionally based on the culture area of the cell culture vessel. If transfected with oligonucleotides or RNA, the conditions for DNA transfection can be referred to.

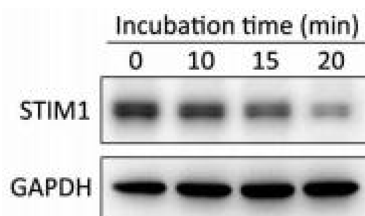

Figure 3.The effect of different incubation times between LipoRibo Transfer Reagent and siRNA on the downregulation of target genes. After incubating LipoRibo Transfer Reagent with STIM1 siRNA for the time shown in the incubation diagram, Hela cells were transfected. After 48 hours, cells were collected and Western blot was used to detect the downregulation effect of STIM1 protein. The STIM1 antibody (AF2614 Stromal interaction molecule 1 Rabbit Monoclonal Antibody) used in Western blot was diluted at 1:1000 according to the instructions, and the secondary antibody horseradish peroxidase labeled goat anti rabbit IgG (H+L)

(A0208) was diluted at 1:1000. GAPDH is an internal reference.

4. Whether it is adherent cells or suspended cells, add the amount of LipoRibo Transfer Agent siRNA mixture evenly to the entire well according to the dosage of 125ul per well in the six well plate, and then gently mix.
5. After about 2 days of further cultivation, appropriate methods such as qPCR can be used to detect the downregulation effect of siRNA on target genes Western、ELISA、 Reporting genes, etc.

Frequently problem:

1. Low transfection efficiency:
    - 1.1. Optimize the ratio of small nucleic acids to LipoRibo Transfer Reagent, and for cells that are difficult to transfect, increase the dosage of LipoRibo Transfer Reagent appropriately.
    - 1.2. High purity, sterile, and pollution-free small nucleic acids such as siRNA should be used for transfection.
    - 1.3. When transfecting adherent cells, they are in good condition and can only be transfected when the cell density reaches 70-80%. Transfection efficiency may be affected by being too thin or too dense, and the optimal transfection density for different cells needs to be explored independently. Suspension cells should be transfected during the logarithmic growth phase.
    - 1.4. A mixture of LipoRibo Transfer Reagent and small RNAs such as siRNA needs to be prepared using antibiotic free and serum free culture media.
    - 1.5. Insufficient cultivation time after transfection was mistaken for low transfection efficiency. The cultivation time required from transfection of different cells to significant expression is usually about 48 hours.
    - 1.6. Check for Mycoplasma infection in cells, which can affect cell proliferation and potentially affect transfection efficiency.
    - 1.7. Using cells with relatively fewer passages can have a certain impact on transfection efficiency if the number of cell passages is too high.
    - 1.8. If no changes in the expression level of the target protein are detected, the small RNA used for transfection should be carefully checked to ensure that the sequencing results and reading frames are completely correct.
    - 1.9. If the knockdown effect of the target gene is not satisfactory, consideration should be given to designing different siRNAs.
2. There is a certain degree of cytotoxicity:
    - 2.1. Before transfection, the cells should be seeded for at least 18-24 hours.
    - 2.2. Reduce siRNA usage and proportionally reduce LipoRibo Transfer Agent.
    - 2.3. Check if the cell density is too low during transfection.
    - 2.4. Check for microbial contamination such as mycoplasma in the cells.
    - 2.5. Change the medium after 4-6 hours of cell culture.

Appendix:

The relevant data tables for the size, culture area, cell culture volume, and recommended culture volume of commonly used porous plates and culture dishes are as follows:

Multiple Well Plates or Dishes	Single Well Only for Plates					
	Diameter (Bottom, mm)*	Growth Area (cm2)*	Average Cell Yield	Total Well Volume (ml)	Working Volume (ml)	Recommended Volume (ml)
6 well	34.8	9.5	9.5×10^5	16.8	1.9-2.9	2
12 well	22.1	3.8	3.8×10^5	6.9	0.76-1.14	1
24 well	15.6	1.9	1.9×10^5	3.4	0.38-0.57	0.5
48 well	11.0	0.95	9.5×10^4	1.6	0.19-0.285	0.25
96 well	6.4	0.32	3.2×10^4	0.36	0.10-0.20	0.1
384 well	2.7	0.056	5.6×10^3	0.112	0.025-0.050	0.030
1536 well	1.63×1.63**	0.025	2.5×10^3	0.0125	0.005-0.010	0.010

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3.5 cm dish	34	9	9.0×10^5	NA	1.8-2.7	2
6 cm dish	52	21	2.1×10^6	NA	4.2-6.3	5
10 cm dish	8.4	55	5.5×10^6	NA	11-16.5	12
15cm dish	14	152	1.5×10^7	NA	30.4-45.6	35
24.5cm dish	22.4× 22.4**	500	5.0×10^7	NA	100-150	120

***Diameter and growth area may vary depending on the manufacturer, and the listed sizes are from Corning.**

****These wells or dishes are square.**