# Instructions for Use of siRNA Transfection Reagent

## Product Information

**Product Name:** OligoMaxi™ RNA Transfection Reagent
**Catalog Number:** R-OM Series (R-OM01 to R-OM04)
**Purchasing link:** https://tarmart.net/reagent/sirna-transfection-reagant#

**Storage and Transport Conditions:** Store at 4°C, transport on ice
**Specification:** 5/10/50/100 mL
**Shelf Life:** 1 year

### siRNA Selection Guide

**PromiseDown™ siRNA Oligo** provides expertly designed oligonucleotides for unmatched specificity and efficiency, ensuring precise and reliable gene silencing. With a broad selection of targets and flexible customization options, we help you push the boundaries of genetic research.

Click here to check your siRNA Oligo: https://tarmart.net/oligo

## Product Introduction

OligoMaxi™ RNA Transfection Reagent is a highly effective siRNA/miRNA transfection reagent that efficiently transfects small RNA molecµles into mammalian cells. It is particµlarly noted for its high transfection efficiency and reproducibility.

In various cell types (such as HeLa, MCF7, or NIH-3T3), OligoMaxi™ RNA Transfection Reagent can achieve over 90% transfection efficiency with low doses of siRNA.

For difficµlt-to-transfect cells, such as K562 and THP-1, OligoMaxi™ RNA Transfection Reagent can still achieve approximately 80% transfection efficiency.

## Product Advantage

1. High transfection efficiency, reproducibility, and simple operation.

2. No need for serum-free media: OligoMaxi™ RNA Transfection Reagent performs well without the need for serum-free media, making it suitable for many cell types.

3. OligoMaxi™ RNA Transfection Reagent works well with difficµlt-to-transfect cell lines, offering stable and reliable transfection resµlts in various cell types.

## Transportation and Preservation

Before use, perform a brief low-speed centrifµgation (recommended speed 1000rpm), and prepare a 20µM storage solution using RNase-free $H_2O$ or sterilized dd$H_2O$. Dispense and store to avoid repeated freeze-thaw cycles (preferably not exceeding five times).

For the list of siRNA products, click to check: PromiseDown™ siRNA Oligo

Table 1. Preparation method for the 20 µM storage solution.

| siRNA(nmol) | 2.5 | 5 | 10 | 50 |
|---|---|---|---|---|
| Dissolution Volume (µl) | 125 | 250 | 500 | 2500 |

***Note:*** *1OD duplex=2.5nmols=40µg*

***Usage Notes:***

*1) siRNA is lightly adhered to the walls of the tube in a dry film form. Before opening the tube, centrifµge it first, and then slowly open the lid. When dissolving, please add sufficient RNase-free $H_2O$ or sterilized dd$H_2O$, then close the lid and gently shake to dissolve.*

*2 ) To avoid degradation of the product caused by external factors (including enzymes, extreme pH, or temperature conditions, etc.), all operations must strictly adhere to RNA handling protocols. During the experiment, it is advisable to keep the product on ice, and after use, please store it carefµlly at -20℃ to -80℃.*

*3 ) siRNA labeled with fluorescent markers (such as CY3 or FAM) may easily experience quenching of fluorescence; therefore, it is essential to take precautions to avoid light exposure during use.*

## Cellµlar Experimental Methods

To minimize variations between wells caused by factors such as cell density, reagent usage, and transfection efficiency, and to ensure the reliability and reproducibility of the experiment, GeneMedi recommends:

1 ) For transfection experiments, ensure that each transfection sample is set up with at least three replicate wells.

2 ) Experimental Group Setup for Transfection:

- Blank control group (required, without transfection reagents and siRNA)
- Transfection reagent control group (required)
- Negative control siRNA group (required)
- Experimental siRNA group (required)
- Fluorescence negative control siRNA group (optional)
- Positive control siRNA group (optional)

When plating cells, ensure that the number of cells seeded in each well is consistent, and try to distribute the cells evenly across the surface of the well.

***Note:*** *The roles of each group in the transfection experiment described in point 2) are detailed at the end of the document.*

## 1. Transfection concentration:

1）In order to achieve optimal gene silencing resμlts, the amount of siRNA transfected into each cell line needs to be experimentally determined. If you are transfecting your cell line for the first time, it is recommended to try using different concentrations of GeneMedi OligoMaxi™-RNA transfection reagent and to vary the concentration of siRNA within the range of 10-100 nM to determine the conditions necessary to achieve the best gene silencing level. High concentrations of siRNA may exhibit cell line dependency.

2）Transfection shoμld be performed when cells are at 30-50% confluence. Generally, gene silencing analysis shoμld be conducted at least 24-72 hours post-transfection. Low-density transfected cells allow for a longer interval between transfection and analysis, reducing cellμlar damage due to overgrowth. Depending on the characteristics of the target gene, high-density transfected cells may be more suitable for optimizing conditions.

3）Do not add antibiotics to the medium during transfection, as this will reduce transfection efficiency and may cause cell death.

4)To achieve better resμlts, it is advisable to dilute the GeneMedi OligoMaxi™-RNA transfection reagent and siRNA in Invitrogen's Opti-MEM low serum medium (or serum-free medium) before forming the complexes. Using fluorescently labeled siRNA can assist in optimizing the transfection conditions for the cell line. Once the optimal conditions for transfection are determined, fluorescently labeled siRNA can be included in each experiment as an indicator of transfection efficiency.

## 2. Transfection:

1）Cell Plating:
Plate cells in the appropriate culture plates one day before transfection to achieve 30-50% confluence at the time of transfection, as shown in Table 2. For optimal transfection of suspension cells using OligoMaxi™-RNA, it is advisable to reduce the volume of the culture medium on the day of transfection compared to conventional culture conditions. Semi-liquid transfection methods can be employed. For various culture plate sizes, the recommended cell number and complete medium volume are provided in Table 3.

**Table 2. Recommended Cell Seeding Numbers One Day Before Transfection (Adherent Cells)**

| Plate Specifications | Seeding Cell Number | Surface Area per Well | Media Volume |
|---|---|---|---|
| 96-well | 5 000 ± 2 500 | 0.3cm$^2$ | 0.2 ml |
| 24-well | 25 000 ± 10 000 | 1.9 cm2 | 0.5 ml |
| 12-well | 50 000 ± 20 000 | 3.8 cm$^2$ | 1 ml |
| 6-well | 150 000 ± 50 000 | 9.4 cm$^2$ | 2 ml |
| 6 cm culture Dish / 25 cm² Flask | 400 000 ± 100 000 | 25 - 28 cm$^2$ | 5 ml |
| 10 cm culture Dish / 75 cm$^2$ Flask | 1 x 10$^6$ ± 250 000 | 75 - 78.5 cm$^2$ | 10 ml |
| 14 cm culture Dish / 175 cm$^2$ Flask | 2 x 10$^6$ - 5 x 10$^6$ | 153 - 175 cm$^2$ | 20 ml |

**Table 3. Seeding Cell Numbers for Suspension Cells on the Day of Transfection**

| Plate Specifications | Seeding Cell Number | Media Volume |
|---|---|---|
| 96-well | 10 000 - 20 000 | 50 µl |
| 24-well | 100 000 - 200 000 | 150 µl |
| 12-well | 200 000 - 400 000 | 300 µl |
| 6-well / 3.5 cm culture Dish | 500 000 - 2 x $10^6$ | 800 µl |

2）Transfection steps

**A**. Adherent cells

To improve transfection efficiency, it is recommended that siRNA concentrations range from 10 nM to 100 nM, and that the amount of OligoMaxi™-RNA shoµld be matched to both siRNA concentration and the specifications of the culture plate. For example, GeneMedi GeneMedi transfected siRNA in a 24-well plate with a transfection concentration of 10 nM, please refer to Table 4 for transfection in other plate sizes.

① Dilute 0.25 µl of the 20 µM siRNA duplex into 100 µl of serum-free medium or Opti-MEM. Gently mix by pipetting up and down 3-5 times.

② Add 0.75 µl of OligoMaxi™-RNA to the 100 µl of siRNA-containing medium described above. Immediately vortex for 10 seconds to mix.

③ Incubate for 10 minutes at room temperature to form a transfection complex between siRNA duplex and GeneMedi OligoMaxi™-RNA, and incubate for no more than 30 minutes.

④ During the formation of the transfection complex, aspirate the original medium from the culture wells and add 0.4 ml of pre-warmed fresh serum-containing complete medium to each well.

⑤ Add 100 µl of the transfection complex incubated in step③ to the wells to be transfected after the fluid change, and gently shake the cell plate to mix. The final volume of medium in each well is 500 µl and the final siRNA concentration is 10 nM.

⑥ The transfected cell plates were incubated in a 5% $CO_2$ incubator at 37°C.

⑦ Gene silencing effect can be analyzed by qPCR (generally recommended 24-48 hours post-transfection) and Western Blot (generally recommended 48-72 hours post-transfection).

***Note:*** *For sensitive cell lines, it is recommended to change the medium 4-6 hours post-transfection.*

**Table 4. Recommended Transfection Conditions For Adherent Cells**

| Plate Specifications | siRNA Final Concentration | siRNA Quantity/Mass Of The Substance | Complete Medium Volume | No Serum Medium Volume | culture Medium Total Volume | 20um Mother Liquid Usage | OligoMaxi™-RNA Volume |
|---|---|---|---|---|---|---|---|
| 96-well | 10-100nM | 2-20pmol /0.032-0.32μg | 150 μl | 50 μl | 200 μl | 0.1-1 μl | 0.2-3 μl |
| 24-well | 10-100nM | 5-50 pmol /0.08-0.8μg | 400 μl | 100 μl | 500 μl | 0.25-2.5μl | 0.5-7.5 μl |
| 12-well | 10-100nM | 10-100 pmol /0.16-1.6μg | 800 μl | 200 μl | 1 ml | 0.5-5 μl | 1-15 μl |
| 6-well | 10-100nM | 20-200 pmol /0.32-3.2μg | 1.8 ml | 200 μl | 2 ml | 1-10 μl | 2-30 μl |
| 6 cm | 10-100nM | 50-500 pmol /0.8-8μg | 4.6 ml | 400 μl | 5 ml | 2.5-25 μl | 5-75 μl |
| 10 cm | 10-100nM | 100-1000pmol /1.6-16μg | 9.5 ml | 500 μl | 10 ml | 5-50 μl | 10-150 μl |

**B.** Suspension cells

For suspension cells, to achieve a higher transfection efficiency, it is recommended to explore transfection with a high siRNA concentration range of 20 nM to 100 nM, while appropriately increasing the usage of the transfection reagent OligoMaxi™-RNA. Reference data for different conditions are provided in Table 5. The following steps outline transfection using 100nM siRNA duplex on a 24-well plate.

① Add 1.25 μl of 20 μM siRNA duplex into 100 μl of serum-free medium or Opti-MEM, and gently pipette up and down 3 to 5 times to mix.

② Add 3.75 μl of OligoMaxi™-RNA to the 100 μl of siRNA-containing medium. Immediately vortex for 10 seconds to mix.

③ Incubate at room temperature for 10 minutes to allow the siRNA duplex and OligoMaxi™-RNA to form a transfection complex. The incubation time shoμld not exceed 30 minutes.

④ Add 100 μl of the transfection complex from step③ to 150 μl of the cell suspension in serum-containing complete medium in the transfection wells. Gently shake the cell plate in a back-and-forth motion to mix. The final volume of the medium in each well is 250 μl, yielding a final concentration of 100 nM siRNA. Add an additional 250 μl of medium 6 hours post-transfection.

⑤ After transfection, the cell plates were cultured in a 37°C, 5% $CO_2$ incubator.

⑥ Gene silencing effect can be analyzed by qPCR (generally recommended 24-48 hours post-transfection) and Western Blot (generally recommended 48-72 hours post-transfection).

***Note:*** *For sensitive cell lines, it is recommended to change the medium 4-6 hours post-transfection.*

**Table 5. Recommended OligoMaxi™-RNA Volumes based on siRNA Concentration and culture Plate Specifications.**

| Plate Specifications | siRNA Final concentration | siRNA quantity/mass of the substance | Cell suspension volume | No serum medium volume | culture medium Total volume | 20uM mother Liquid usage | OligoMaxi™ -RNA volume | 6h supplement liquid volume |
|---|---|---|---|---|---|---|---|---|
| 96-well | 20-100nM | 2-10pmol /0.032-0.16μg | 50 μl | 50 μl | 100 μl | 0.1-0.5 μl | 0.2-1.5 μl | 100 μl |
| 24-well | 20-100nM | 5-25 pmol /0.08-0.4μg | 150 μl | 100 μl | 250 μl | 0.25-1.25μl | 0.5-3.75 μl | 250 μl |
| 12-well | 20-100nM | 10-50 pmol /0.16-0.8μg | 300 μl | 200 μl | 500 μl | 0.5-2.5 μl | 1-7.5μl | 500 μl |
| 6-well | 20-100nM | 20-100 pmol /0.32-1.6μg | 800 μl | 200 μl | 1 ml | 1-5 μl | 2-15 μl | 1 ml |
| 6 cm | 20-100nM | 50-250 pmol /0.8-4μg | 2 ml | 500 μl | 2.5 ml | 2.5-12.5 μl | 5-37.5 μl | 2.5 ml |

## 3. Frequently asked questions (FAQ):

| Issue | Suggestions for resolution |
|---|---|
| Low silencing efficiency | Increase the siRNA concentration in each well |
| | Increase the amount of GeneMedi OligoMaxi™-RNA transfection reagent in each well |
| | The silencing efficiency at different time points of transfection 24h~96h was detected |
| | Dilute siRNA with opti-MEM |
| | Ensure that adherent cells are 30-50% confluent on the day of transfection; For small cells and slow-growing cell types, seed approximately 2 mL per well |
| | Confirm that all reagents are RNase-free |
| | Ensure siRNA is of high quality (PAGE purification and desalting) |
| | Confirm the concentration and annealing conditions of the siRNA duplex |
| | Reduce the volume of media during transfection by half and centrifuge the plate at low speed (180 g, 5 min). After 4 h, add 0.5 ml of medium |
| Cytotoxicity | Reduce the incubation time of the OligoMaxi™-RNA/siRNA complex with the cells by changing the medium 4 to 6 hours after transfection or simply adding fresh medium to the medium |
| | Reduces the volume of GeneMedi OligoMaxi™-RNA used in transfection tests |
| | Verify whether silencing the target gene affects cell viability |

## Attach

**Roles of Each Group in the Experimental Setup:**

1） Blank control group (required, without transfection reagents and siRNA)
The target cells without any transfection treatment are used to observe the growth status of the cells during the whole experiment and the reference of subsequent related detection experiments.

2） Transfection reagent control group (required)
Transfect the cells of interest with only the transfection reagent without the addition of siRNA to observe whether the transfection reagent has a potential toxic effect on the cells.

3） Negative control siRNA group (required)
Transfected with siRNA-negative control (siRNA NC) group, the siRNA-negative control did not target any gene, and served as a strict control group for the siRNA group of target genes to illustrate the specificity of siRNA action.

4）Experimental siRNA group (required)
This group is transfected with the siRNA targeting the gene of interest. It is used to knock down the expression of the target gene.

5） Fluorescence negative control siRNA group (optional)
Transfection of fluorescently labeled siRNA-negative controls differs from negative control siRNAs only in fluorescent labeling, which is used to indicate transfection efficiency, and it is recommended that this group be included in every experiment. (CY3: excitation light 550nm, emission light 570nm; FAM: Excitation light 492nm, emission light 518nm.）

6) Positive control siRNA set (optional)
This group is transfected with siRNA targeting a housekeeping gene (e.g., GAPDH) or a reporter gene (e.g., luciferase), which has been validated for effective silencing. This group helps verify the transfection efficiency and the accuracy of the detection methods by evaluating the knockdown efficiency.

**The positive control siRNAs available from GeneMedi are as follows:**

| Species | Target genes |
|---|---|
| person | GAPDH、LaminA/C、Beta-Actin、MAPK1 |
| Mice | MAPK1 |
| Rat | MAPK1 |
| reporter genes | GFP、Luciferase GL2、Luciferase GL3 |