3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

MISSION® siRNA Transfection Reagent

Catalog Number **S1452**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

MISSION® siRNA Transfection Reagent has been designed to deliver small RNA (siRNA and miRNA) in a wide variety of mammalian cell lines. High silencing efficiency can be achieved using a low amount of siRNA.

MISSION microRNA Mimic concentrations of 10–100 nM have been used successfully in a variety of cell lines, but lower or higher concentrations may be necessary for specific applications. Please refer to the MISSION microRNA Mimics (Catalog Number MI00200) bulletin for preparation instructions.

MISSION siRNA Transfection Reagent is provided as a sterile solution and is compatible with serum and antibiotics.

For 1.0 mL of MISSION siRNA Transfection Reagent, this table provides the number of reactions for various plate sizes:

Plate size	Volume of siRNA transfection reagent per well (µL)	Reactions (1.0 mL tube)
96 well	1–3	300–1,000
24 well	2–5	200–500
12 well	4–10	100–250
6 well	8–16	60–125

Precautions and Disclaimer

For R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

MISSION siRNA Transfection Reagent is shipped on wet ice. Upon receipt, store at 2–8 °C. **Do not freeze**. Make sure to always tighten the cap on the tube prior to storing. Stored under these conditions, the product remains stable for one year.

Procedures

I. **Transfection protocol for adherent cells**
Follow this protocol as a starting point and further optimize the experiments using the guidelines given at the end of the protocol, especially if this is the first time for transfecting a cell line with siRNA or miRNA.

A. Forward transfection of siRNA into adherent cells
For optimal transfection conditions with MISSION siRNA Transfection Reagent, the cells should be 30–50% confluent on the day of transfection. For 24-well plates, splitting the cells and seeding 15,000-35,000 cells per well 24 hours before transfection is suggested. For other plate sizes, see Table 1 to adjust the number of cells.

Testing siRNA concentrations ranging from 1–50 nM to identify optimal siRNA transfection conditions is recommended. See Table 2 for the amount of siRNA needed to achieve the desired concentration in different plate sizes.

For 1 nM siRNA concentration, using 1–3 µL of MISSION siRNA Transfection Reagent per well in a 24-well plate is recommended. For siRNA or miRNA concentrations above 10 nM, using 2–4 µL of MISSION siRNA Transfection Reagent per well in a 24-well plate is recommended. Please refer to Tables 1 and 2 for volume guidelines for additional plate sizes and concentrations.

Table 1.
Protocol conditions for transfection of adherent cells for different plate sizes and different siRNA concentrations.

Culture vessel (wells)	Number of adherent cells to seed	Volume of medium to seed cells (mL)	Volume of siRNA transfection reagent (µL) for siRNA conc. 1-10 nM	Volume of siRNA transfection reagent (µL) for siRNA conc. >10 nM	Volume of transfection mix (serum-free medium)	Volume of complete medium	Final volume
384	1,250–3,750	0.1	0.25 – 0.6	0.35 – 0.75	15 µL	45 µL	60 µL
96	2,500–7,500	0.2	0.5 – 1.2	0.7 – 1.5	50 µL	125 µL	175 µL
24	15,000–35,000	1	1–3	2 – 4	100 µL	500 µL	600 µL
12	30,000–70,000	2	2–6	4 – 8	200 µL	1 mL	1.2 mL
6	100,000–200,000	4	4–12	8 – 16	200 µL	2 mL	2.2 mL

Table 2.
Quantity of siRNA to dilute in serum free-medium to obtain a 1-50 nM final concentration for different plate sizes for adherent cells transfection.

	Amount of siRNA (pmol) for various final concentrations				
Culture vessel (wells)	1 nM	5 nM	10 nM	25 nM	50 nM
384	0.06	0.3	0.6	1.5	3
96	0.17	0.85	1.7	4.25	8.5
24	0.6	3	6	15	30
12	1.2	6	12	30	60
6	2.2	11	22	55	110

The following protocol is given for transfection in 24-well plates at 10 nM siRNA concentration. All amounts and volumes are given on a per well basis

1. Dilute 6 pmol of the siRNA duplex into 100 µL of serum-free medium. Homogenize gently.
2. Add 2 µL of MISSION siRNA Transfection Reagent into the 100 µL of the diluted siRNA.
3. Mix the solution immediately and incubate for 10–15 minutes at room temperature.
   Note: Proceed to Step 5 within 30 minutes.
4. During the incubation time, remove the culture medium and add 500 µL of fresh pre-warmed medium with serum.
   Note: MISSION siRNA Transfection Reagent is compatible with antibiotics.
5. Add the MISSION siRNA Transfection Reagent/ siRNA solution onto the cells and homogenize the mixture by gently rocking the plate. The final volume is 600 µL.
6. Incubate the plate at 37 °C.
7. Gene silencing is usually measured 24–96 hours after transfection.
   Note: If longer incubation times are required, ensure the cells are always covered by sufficient culture medium and replace medium as required.

B. Reverse transfection of siRNA into adherent cells
In this procedure, transfection and cell plating are performed on the same day. siRNA is diluted in bulk and distributed into each well along with MISSION siRNA Transfection Reagent prior to the addition of the adherent cells. During the incubation time, cells are split and diluted to the desired concentration and then added into each well containing MISSION siRNA Transfection Reagent/ siRNA solution.

For such experiments, cells are seeded according to plate size, see Table 3.

Table 3.
Number of cells to seed per well on the day of transfection

Culture vessel	Number of cells	Volume of medium (µL)
384-well	2000–3000	45
96-well	5,000–10,000	125
24-well	30,000–50,000	500

Table 4.
Protocol conditions for reverse transfection of adherent cells for different plate sizes at 10 nM siRNA. For other siRNA concentrations, please refer to Tables 1 & 2.

Culture vessel	Amount of siRNA (pmol)	Volume of transfection mix (serum-free medium)	Volume of transfection reagent (µl)	Volume of complete medium (µl)	Final volume (µl)
384 well	0.6	15 µl	0.25–0.75	45	60
96 well	1.7	50 µl	0.25–1.25	125	175
24 well	6	100 µl	1–3	500	600

The following protocol is given for reverse transfection in 96-well plates at 10 nM final siRNA concentration.

1. Prepare a master-mix by diluting 1.7 pmol of siRNA duplexes in 50 µL of serum-free medium for each well to be used in the experiment. Homogenize gently by pipetting up and down.
2. Add 50 µL of master mix per well onto each well.
3. Add 1 µL of MISSION siRNA Transfection Reagent into each well.
   Note: For automated dispensers, dilute MISSION siRNA Transfection Reagent 1:5 in water and add 5 µL of the diluted solution per well. Do not store the diluted MISSION siRNA Transfection Reagent for more than 24 hours.
4. Immediately mix by placing the plate on a shaker for 3–5 minutes, or pipetting up and down, and incubate for 10–15 minutes.
   Note: Proceed to Step 5 within 30 minutes.
5. Add 7,500 cells in 125 µL of complete medium per well and homogenize the mixture by gently rocking the plate. The final total volume is 175 µL.
6. Incubate the plate at 37 °C.
7. Gene silencing is usually measured 24–96 hours after transfection.
   Note: If longer incubation times are required, ensure the cells are always covered by sufficient culture medium and replace medium as required.

II. **Forward transfection of suspension cells**
For optimal transfection conditions of suspension cells with MISSION siRNA Transfection Reagent, cells should be diluted to the desired amount on the day of transfection (refer to Table 5 for the recommended number of cells to seed and the total volume of transfection mix).

The following protocol is given for transfection in 24-well plates at the siRNA concentration of 10 nM. All amounts and volumes are given on a per well basis (refer to Tables 5 and 6 for transfection conditions in other plate sizes).

1. Dilute 3 pmol of siRNA duplexes into 100 µL of serum-free culture medium. Homogenize gently.
2. Add 3 µL of MISSION siRNA Transfection Reagent into the diluted siRNA solution. Mix immediately for 10 seconds.
3. Incubate for 10–15 minutes at room temperature.
   Note: Proceed to Step 4 within 30 minutes.
4. Add the 100 µL of transfection mix onto the cells in medium with serum and homogenize gently. The final siRNA concentration is 10 nM in a total volume of 300 µL.
5. Incubate the plate at 37 °C.
6. Add 0.7 mL of complete medium after 4–6 hours.
7. Gene silencing is usually measured 24–96 hours after transfection. For this purpose, the cells growing in suspension are collected by centrifugation at 400 × *g* and resuspended in the required medium or buffer
   Notes: If longer incubation times are required, ensure the cells are always covered by sufficient culture medium and replace medium as required.

   After 4-6 hours, add the volume of medium per well indicated in Table 5.

Table 5.
Protocol conditions for transfection of suspension cells for different plate sizes and different siRNA concentrations.

Culture vessel	Number of suspension cells to seed	Volume of medium to seed cells	Volume of siRNA transfection reagent (µL) for siRNA conc. 1-10 nM	Volume of siRNA transfection reagent (µL) for siRNA conc. >10 nM	Volume of transfection mix (µL)	Volume of medium to add after 4–6 hours
384-well	5,000–10,000	25 µL	0.5–1.5	1–2	25	0 µL
96-well	10,000–20,000	50 µL	1–3	2–4	50	100 µL
24-well	100,000–200,000	200 µL	1–5	3–7	100	0.7 mL
12-well	200,000–400,000	500 µL	2–10	4–14	200	1 mL
6-well	500,000–2,000,000	1 mL	2–18	7–23	200	2 mL
60 mm/ flask 25 cm^2	2,000,000–5,000,000	2 mL	5–25	14–46	400	4 mL

Table 6.
Quantity of siRNA to dilute in serum free-medium to obtain a 1-50 nM final concentration for different plate sizes for suspension cells transfection.

	Amount of siRNA (pmol) for various final concentrations				
Culture vessel	**1 nM**	**5 nM**	**10 nM**	**25 nM**	**50 nM**
384-well	0.05	0.25	0.5	1.25	2.5
96-well	0.15	0.5	1.5	2.5	5
24-well	0.3	1.5	3	7.5	15
12-well	0.6	3	6	15	30
6-well	1.2	6	12	30	60
60 mm/ flask 25 cm^2	2.4	12	24	60	120

Transfection efficiency
MISSION siRNA Transfection Reagent is not affected by the presence of serum during transfection. Therefore, the transfection mix can be added directly to the medium with serum. Transfection efficiency can be improved by using smaller volumes of medium and/or by centrifugation of the culture plate (5 minutes at 280 × *g* at room temperature). If cytotoxicity is observed, it is suggested to change the medium after 2–4 hours to stop the transfection. Complete medium can also be added to each well without removing the transfection mix in order to dilute the reagent and reduce potential cytotoxicity.

Optimization guidelines
Improving transfection efficiency
- Use Sigma's Pre-designed or Custom siRNA duplexes designed using our Rosetta design algorithm. Use Sigma's miRNA mimics for miRNA transfection
- Check that all reagents used in the experiment are RNase-free.
- Decrease the volume of culture medium used during transfection.
- Ensure that adherent cells are 30–50% confluent on the day of transfection.
- Optimize the volume of MISSION siRNA Transfection Reagent used during transfection.

Reducing cellular toxicity
- Reduce the incubation time of the transfection mix with the cells.
- Reduce the volume of the MISSION siRNA Transfection Reagent used.
- Check that the silencing of the targeted gene is not affecting cell viability.

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