SKU # SL100568 Store at 4 °C

GenMute™ siRNA Transfection Reagent

----- A General Protocol for Transfecting siRNA to Mammalian Cells

- ☐ 100 µL
- ☐ 500 µL
- ☐ 1000 µL

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This product is for laboratory research ONLY and not for diagnostic use

Introduction

GenMute™ siRNA Transfection Reagent is a biodegradable, polymer-based transfection reagent engineered for efficient intracellular delivery of small interfering RNA (siRNA) and plasmid DNA. The reagent incorporates proprietary pH-Dependent Conformational Change (PDCC) technology, whereby the polymer backbone is chemically functionalized with pre-screened hydrophobic moieties on its side chains. This modification enables endosomal escape and facilitates nucleic acid release into the cytoplasm. GenMute™ Reagent has been validated for transfection of single-species nucleic acids (siRNA, miRNA mimics, or plasmid DNA) as well as co-transfection of DNA/siRNA or DNA/miRNA mimic combinations across a broad range of mammalian cell types.

Important Guidelines

- Use of GenMute™ Transfection Buffer working solution (1×) as the diluent for both nucleic acid and reagent preparations is **required** for maximum gene silencing efficacy.
- The standard protocols below represent recommended starting conditions; optimization of reagent-to-nucleic acid ratios and incubation times may be necessary for specific cell lines or experimental objectives.
- For standard plasmid DNA transfection or reverse siRNA transfection protocols, refer to https://signagen.com

PART I. Standard siRNA Transfection of Adherent Cells

Step 1. Preparation of Working Solution of GenMute™ Transfection Buffer (1x):

GenMute™ Transfection Buffer (5x) is provided as 5x concentrated stock solution. To make working solution (1x), dilute one part of the stock solution with 4 parts of ddH_2O into a sterile bottle. The working solution is stable at RT for 24 months.

Note: *Always keep GenMute™ Transfection Buffer (5x) at RT. If refrigerated, white precipitates may appear. It won't affect the transfection efficiency. After dilution with 4 parts of ddH_2O to make GenMute™ Transfection Buffer (1x) working solution, the white precipitates will disappear. Always keep GenMute™ Transfection Buffer working solution (1x) at RT.*

Table 1. General Guidelines for siRNA Transfection According to Cell Culture Vessel Format

Culture Dish	Growth Medium (mL)	Transfection Buffer (1x) (µL)	siRNA (pmol) Final 5.0 or 50 nM	GenMute™ Reagent (µL)
24-well	1	50	5.0 / **50**	1.2 ~ **2.0**
12-well	1.5	75	7.5 / **75**	2.0 ~ **3.3**
6-well	2	100	10 / **100**	2.4 ~ **4.0**
60 mm	5	300	25 / **250**	7.2 ~ **12**
10 cm / Flask 75	10	600	50 / **500**	14 ~ **24**

Step 2. Cell Seeding:

Seed cells 18–24 hours prior to transfection to achieve approximately 50% confluency at the time of transfection. Replenish each well with fresh complete growth medium (containing serum and antibiotics) 30–60 minutes before initiating the transfection procedure.

Note: GenMute™ Reagent is compatible with serum- and antibiotic-containing media and does not require serum-free conditions at any stage of the experiment.

Step 3. siRNA Transfection

A final siRNA concentration of 1–100 nM is recommended. For most adherent cell lines and primary cells, a starting concentration of **5 nM** typically yields effective silencing. For difficult-to-transfect cell types, a concentration of **50 nM** is recommended (indicated in bold/underline in **Table 1**).

The following protocol is described per well of a 6-well plate. For other culture vessel formats, refer to **Table 1**.

1. Add 2.0 mL of fresh complete medium (with serum and antibiotics) to each well 30–60 minutes before transfection.
2. Dilute 10 pmol (for 5 nM final) or 100 pmol (for 50 nM final) siRNA into 100 µL of GenMute™ Transfection Buffer (1×). Vortex briefly to mix.
   Note: Prepare siRNA stocks at 10 µM; add 1.0 µL (for 5 nM) or 10 µL (for 50 nM) per well accordingly. Use of GenMute™ Transfection Buffer (1×) as the diluent is essential for optimal silencing.
3. Add 2.4 µL GenMute™ Reagent (or 4.0 µL for difficult-to-transfect cells). Mix by pipetting up and down gently.
4. Incubate the siRNA–reagent mixture at RT for approximately 15 minutes to allow transfection complex formation.
   Note: Do not extend complex incubation beyond 30 minutes, as this may reduce transfection efficiency.
5. Add the transfection complex dropwise to the cells. Rock the plate gently and return the plate to the CO_2 incubator.
6. If required, replace the transfection medium with fresh complete growth medium approximately 5 hours post-transfection.
7. Assess gene silencing by qRT-PCR, Western blot, or functional assay 24–72 hours post-transfection.

PART II. A Standard Protocol for DNA/siRNA Co-transfection

Step 1. Preparation of GenMute™ Transfection Buffer Working Solution (1×)

Prepare as described in Part I, Step 1.

Step 2. Cell Seeding

Seed cells 18–24 hours prior to transfection to achieve approximately 70% confluency at the time of transfection.

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Add fresh complete growth medium 30–60 minutes before transfection.
Note: GenMute™ Reagent performs equivalently in the presence of serum and antibiotics; serum-free media substitution is not required.

Step 3. DNA/siRNA Co-transfection

For co-transfection, use 0.3–0.5 µg plasmid DNA and 1–20 nM siRNA per well of a 6-well plate. A recommended starting condition is 0.5 µg DNA and 20 pmol siRNA (10 nM final concentration) per well, which yields satisfactory knockdown for most cell lines.

The following protocol applies per well of a 6-well plate. For other culture formats, refer to **Table 2**.

1. Add 1.0 mL of fresh complete medium (with serum and antibiotics) to each well 30–60 minutes before transfection.
2. Combine 0.5 µg plasmid DNA and 10 pmol siRNA in 100 µL of GenMute™ Transfection Buffer (1×). Mix gently by pipetting.
   Note: Prepare siRNA stocks at 10 µM; add 1.0 µL per well to achieve a 10 nM final concentration. Use of GenMute™ Transfection Buffer (1×) for dilution is critical for co-transfection efficiency.
3. Immediately add 3 µL GenMute™ Reagent and mix by pipetting up and down.
4. Incubate at RT for approximately 15 minutes to allow ternary complex formation.
   Note: Do not exceed 30 minutes of complex incubation.
5. Add the transfection complex dropwise to the cells. Rock the plate gently and return to the CO_2 incubator.
6. Replace the transfection medium with fresh complete growth medium approximately 5 hours post-transfection, if required.
7. Analyze knockdown efficiency 24–72 hours post-transfection by appropriate molecular or functional assays.

Table 2. General Guidelines for DNA and siRNA Co-Transfection According to Cell Culture Vessel Format

Culture Dish	Growth Medium (mL)	Transfection Buffer (1x) (µL)	Plasmid DNA (µg)	siRNA (pmol) Final 10 nM	GenMute Reagent (µL)
24-well	1.0	50	0.25	10	1.5
12-well	1.5	75	0.375	15	2.25
6-well	2.0	100	0.5	20	3
60 mm	5.0	300	1.5	50	7.5
10 cm / flask 75	10	600	4.0	100	15

Storage: GenMute™ siRNA Transfection Reagent is stable for up to **24 months at 4° C**. GenMute™ Transfection Buffer (5 x) and (1x) must be stored at RT. The product is shipped at ambient temperature.