Cat # 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™ Reagent is a novel biodegradable polymer based siRNA and DNA transfection reagent. With our proprietary pH Dependent Conformational Change (PDCC) technology, the polymer was chemically modified by addition of pre-screened hydrophobic groups to side chain, making GenMute™ Reagent a versatile and most powerful gene delivery tool. GenMute™ Reagent have been validated to effectively and reproducibly transfect single siRNA, miRNA mimics, DNA or co-transfect DNA/siRNA, DNA/miRNA mimics to variety of mammalian cells.

**Important Guidelines for Transfection:**

- For maximum gene silencing, using GenMute™ Transfection Buffer working solution (1x ) to dilute siRNA/DNA and GenMute™ Reagent is a must.
- While the standard protocols for siRNA transfection and siRNA/DNA co-transfection are being given below, optimization is often needed for maximal gene silencing.
- For a standard DNA transfection or reverse siRNA transfection protocol, please visit the link: www.signagen.com/genmute

**PART I. Standard siRNA Transfection of Adherent Cells**

**Step I. 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.

**Step II. Cell Seeding:**

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal ~50% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 30~60 min before transfection.

**Note:** GenMute™ reagent is NOT interfered by serum and antibiotics, therefore serum and antibiotic containing medium can be used during the entire experiment.

**Table 1. A Guideline for siRNA transfection per cell culture vessel**

| Culture Dish | Growth Medium (mL) | Transfection Buffer (µL) | siRNA (pmoles) Final 5.0 or **50** nM | GenMute™ Reagent (µL) |
|---|---|---|---|---|
| 24-well | 0.5 | 50 | 2.5 / **25** | 1.2 ~ **2.0** |
| 12-well | 0.75 | 75 | 3.75 / **38** | 2.0 ~ **3.3** |
| 6-well | 1.0 | 100 | 5.0 / **50** | 2.4 ~ **4.0** |
| 60 mm | 3.0 | 300 | 15 / **150** | 7.2 ~ **12** |
| 10 cm / Flask 75 | 8.0 | 800 | 40 / **400** | 20 ~ **33** |

**Step III. siRNA Transfection Protocol:**

For optimal siRNA-mediated silencing, we recommend using 1~100 nM siRNA. As a starting point, we recommend using 5.0 nM siRNA which usually gives satisfactory silencing result for most adherent cell lines or primary cells. For hard-to-transfection cells, we recommend using a final siRNA concentration of 50 nM. (bold & underlined in **Table 1**).

The following conditions are given per well in a 6 well plate. For other culture format, please refer to **Table 1**.

- For each well, add 1.0 mL of complete medium with serum and antibiotics freshly 30~60 min before transfection.
- Dilute 5.0 or **50** pmoles siRNA (final concentration of 5.0 or **50** nM respectively per well) into 100 µL of working solution of GenMute™ Transfection Buffer prepared in **Step I**. Vortex to mix.

  **Note: For maximum gene silencing, dilute siRNA and GenMute™ reagent with GenMute™ Transfection Buffer working solution (1x ).**
  **We strongly suggest reconstituting siRNA stock solution at 10 µM, so add 0.5 or 5.0 µL siRNA stock solution per well of 6-well plate to make final 5.0 and 50 nM siRNA respectively.**
- Add 2.4 µL or **4.0 µL** (for hard-to-transfect cells, bold and underlined in **Table 1**) GenMute™ reagent, mix by pipetting up and down.
- Incubate for ~15 min at RT to let transfection complex form. **Note: Never keep the complex longer than 30 min.**
- Add the transfection mix to the cells drop wise. Gently rock the plate back and forth and return the plate to CO2 incubator.
- Replace transfection medium by cell growth medium ~5 hours after transfection when necessary.
- Gene silencing is usually measured 24~72 hours post transfection.

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

**Step I. Preparation of Working Solution of GenMute™ Transfection Buffer:**

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

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# GenMute™ siRNA & DNA Transfection Reagent

----- A General Protocol for Co-transfecting DNA/siRNA to Mammalian Cells

☐ 100 µl
☐ 500 µl
☐ 1000 µl

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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.

**Step II. Cell Seeding:**

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal ~70% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 30~60 min before transfection.

**Note:** GenMute™ reagent is NOT interfered by serum and antibiotics, therefore serum and antibiotic containing medium can be used during the entire experiment.

**Table 2. A Guideline for DNA & siRNA Co-transfection Per Cell Culture Vessel**

| Culture Dish | Growth Medium (mL) | Transfection Buffer (µL) | Plasmid DNA (µg) | siRNA (pmoles) Final 10 nM | GenMute Reagent (µL) |
|---|---|---|---|---|---|
| 24-well | 0.5 | 50 | 0.25 | 5 | 1.5 |
| 12-well | 0.75 | 75 | 0.375 | 7.5 | 2.25 |
| 6-well | 1.0 | 100 | 0.5 | 10 | 3 |
| 60 mm | 3.0 | 300 | 1.5 | 30 | 9 |
| 10 cm / flask 75 | 8.0 | 800 | 4.0 | 80 | 24 |

**Step III. DNA & siRNA co-transfection protocol:**

For DNA/siRNA co-transfection experiment, we recommend using 0.3~0.5 µg DNA and 1~20 nM siRNA per well in a 6-well plate. As a starting point, we recommend using 0.5 µg DNA and 10 pmoles siRNA (final concentration 10 nM) per well of a 6-well plate which usually give satisfactory knockdown effect.

The following conditions are given per well of a 6 well plate. For other culture format, please refer to **Table 2**.

- For each well, add 1.0 mL of complete medium with serum and antibiotics freshly 30~60 min before transfection.
- Dilute 0.5 µg DNA and 10 pmoles siRNA (final 10 nM) into 100 µL of working solution of GenMute™ Transfection Buffer prepared from **Step I**. Mix by pipetting up and down.
  **Note:** For optimal transfection efficiency and maximum gene silencing, GenMute™ Transfection Buffer working solution is a must for diluting siRNA/DNA and GenMute™ reagent. We strongly suggest preparing siRNA stock solution at 10 µM, so add 1.0 µL siRNA stock solution per well of 6-well plate to make final 10 nM of siRNA.
- Add 3 µL GenMute™ reagent immediately, mix by pipetting up and down.
- Incubate for ~15 min at RT to let transfection complex form.

**Note:** Never keep the complex longer than 30 min.

- Add the transfection complex to the cells drop wise.
- Gently rock the plate back and forth and return the plate to the incubator.
- Replace transfection medium by cell growth medium ~5 hours after transfection when necessary.
- Gene silencing is usually measured 24~72 hours post transfection.

**Storage:** GenMute™ siRNA Transfection Reagent is stable for up to 12 months at 4 °C. Always keep GenMute™ Transfection Buffer (5x ) at RT. This item is shipped at ambient temperature.