

GenMute™ siRNA & DNA Transfection Reagent



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

- 100 μ l
- 500 μ l
- 1000 μ l

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 biodegradable 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:

- GenMute™ reagent was formulated as a versatile gene delivery tool. While this protocol gives procedures for co-transfecting siRNA/DNA and for transfecting siRNA to mammalian cells, the protocol for miRNA mimics and DNA transfection can be obtained from our website.
- For maximum gene silencing, we recommend using GenMute™ Transfection Buffer to dilute siRNA/DNA and GenMute™ Reagent. Never use pyruvate or serum containing mediums like DMEM or Opti-MEM to dilute siRNA/DNA and GenMute™ reagent.

1. DNA & siRNA Co-transfection

1.1 Cell Seeding:

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal ~80% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 30~60 minutes 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 DNA & siRNA Co-transfection Per Cell Culture Vessel

Culture Dish	Growth Medium (ml)	Diluent Volume (μ L)	Plasmid DNA (μ g)	siRNA (pmol) Final 5.0 nM	GenMute™ (μ L)
24-well	0.5	50	0.5	2.5	1.5
12-well	0.75	75	0.75	3.25	2.25
6-well	1.0	100	1.0	5.0	3
60 mm	3.0	300	3.0	15	9
10 cm /flask 75	8.0	800	8.0	40	24

1.2 DNA & siRNA co-transfection protocol:

For DNA/siRNA co-transfection experiment, we recommend using

0.5 ~ 1.0 μ g DNA and 1 ~ 20 nM siRNA per well in a 6-well plate. As a starting point, we recommend using 1.0 μ g DNA and 5.0 pmol siRNA (final concentration 5.0 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 1**.

- For each well, add 1.0 ml of complete medium with serum and antibiotics freshly 30~60 minutes before transfection.
- Dilute 1.0 μ g DNA and 5.0 pmoles siRNA (final concentration: 5.0 nM) into 100 μ l of GenMute™ transfection buffer. Mix by pipetting up and down and let it sit at RT for 5 minutes.

Note: For optimal transfection efficiency and maximum knockdown result, use GenMute™ transfection buffer to dilute siRNA/DNA and GenMute™ reagent. Pyruvate and serum interfere formation of the transfection complex. So never use mediums like DMEM which may contain sodium pyruvate and Opti-MEM which contains serum. We strongly suggest preparing siRNA stock solution at 5.0 μ M, so add 1.0 μ l siRNA stock solution per well of 6-well plate to make final 5.0 nM of siRNA.

- Add 3 μ l GenMute™ reagent, vortex briefly.
- Incubate for ~15 min at RT to let transfection complex form. Never keep the complex longer than 20 minutes.
- 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 ~4 hours after transfection when necessary.
- Gene silencing is usually measured 24~72 hours post transfection for mRNA levels and 48~96 hours for proteins.

2. siRNA Transfection

2.1 Cell Seeding:

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal ~40% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 30~60 minutes 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.

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- 100 µl
- 500 µl
- 1000 µl

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2.2 siRNA Transfection Protocol:

For optimal siRNA-mediated silencing, we recommend using 1 ~ 20 nM siRNA. As a starting point, we recommend using 10 nM siRNA which usually gives satisfactory knockdown effect.

The following conditions are given per well in 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 minutes before transfection.
- Dilute 10 pmoles siRNA (final concentration of 10 nM per well) into 100 µl of GenMute™ buffer. Mix by pipetting up and down and let it sit at RT for 5 minutes.

Note: For optimal transfection efficiency and maximum silencing, use GenMute™ transfection buffer to dilute siRNA and GenMute™ reagent. Pyruvate and serum interfere formation of the transfection complex. So never use mediums like DMEM which may contain sodium pyruvate and Opti-MEM which contains serum. We strongly suggest preparing siRNA stock solution at 5.0 µM, so add 2.0 µl siRNA stock solution per well of 6-well plate to make final 10.0 nM of siRNA.

- Add 1.0 µl GenMute™ reagent, vortex and spin down briefly.
- Incubate for ~15 min at RT to let transfection complex form.
- Note: Never keep the complex longer than 20 minutes.**
- Add the transfection mix to the cells drop wise. Gently rock the plate back and forth and return the plate to the incubator.
- Gene silencing is usually measured 24~72 hours post transfection for mRNA levels and 48~96 hours for proteins.
- For optimal silencing, scale down siRNA concentration to 5.0~1.0 nM with proportional amount of GenMute™ Transfection Buffer if you are not satisfied with silencing at 10 nM of siRNA.

Table 2. A Guideline for siRNA Transfection Per Cell Culture Vessel

Culture Dish	Growth Medium (ml)	Diluent Volume (µL)	siRNA (pmol) 10 nM Final	GenMute™ reagent (µL)
24-well	0.5	50	5	0.5
12-well	0.75	75	7.5	0.75
6-well	1.0	100	10	1.0
60 mm	3.0	300	30	3.0
10 cm /flask 75	8.0	800	80	8.0

Storage: GenMute™ siRNA & DNA Transfection Reagent is stable for up to 12 months at 4 °C. This item shipped at ambient temperature