

GenMute™ siRNA & DNA Transfection Reagent



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----- A General protocol for transfecting mammalian cells

- 100 µl
- 500 µl
- 1000 µl

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, DNA or co-transfect DNA/siRNA 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 transfecting DNA to mammalian cells, the protocol for siRNA or siRNA/DNA transfection can be obtained from our website.
- For maximum efficiency, we recommend using GenMute™ Buffer to dilute DNA and GenMute™ Reagent. Alternatively serum free RPMI 1640 medium is acceptable as diluent. Never use serum free DMEM which may contain sodium pyruvate and Opti-MEM which contains serum.

Procedures fro Transfecting DNA to Mammalian Cells

I. Preparation of Working Solution of GenMute™

Transfection Buffer:

GenMute™ Transfection Buffer (5x) is provided as 5x concentrated stock solution. To make working solution, dilute one part of the stock solution with 4 parts of ddH₂O into a sterile bottle. The working solution is stable at 4 °C~RT for 12 months.

II. 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.

Table 1. A Guideline for DNA Co-transfection Per Cell Culture Vessel

Culture Dish	Growth Medium (ml)	Transfection Buffer (µL)	Plasmid DNA (µg)	GenMute™ Reagent (µL)
24-well	0.5	50	0.5	1.5
12-well	0.75	75	0.75	2.25
6-well	1.0	100	1.0	3
60 mm	3.0	300	3.0	9
10 cm/flask	75 8.0	800	8.0	24

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

III. DNA Transfection protocol:

For DNA transfection experiment, we recommend using 0.5 ~ 1.0 µg DNA per well in a 6-well plate. As a starting point, we recommend using 1.0 µg DNA and 3.0 µl GenMute™ reagent per well of a 6-well plate which usually give satisfactory efficeincy without visible toxicity.

- 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 µg DNA into 100 µl of GenMute™ buffer. Mix by pipeting up and down.
 - Add 3 µl GenMute™ reagent, vortex briefly.
 - Incubate for ~10 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 24 hours after transfection and analyze as required.

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