Cat # SL100568-N2A Store at 4 °C

GenMute™ siRNA Transfection Reagent for Neuro-2A

----- A General Protocol for Transfecting siRNA to RAW 264.7

- ☐ 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 biodegradable polymer was chemically modified by addition of pre-screened hydrophobic groups to side chain, making GenMute™ Reagent the most powerful siRNA delivery tool. GenMute™ siRNA Transfection Reagent for Neuro-2A is pre-optimized for transfecting siRNA to Neuro-2A cell with maximum silencing.

Important Guidelines for Transfection:

- For maximum gene silencing, we recommend using GenMute™ Transfection Buffer to dilute siRNA/DNA and GenMute™ Reagent.
- While the standard protocol for siRNA transfection to Neuro-2A is being given below, optimization is sometimes needed for different siRNAs.

Standard siRNA Transfection Protocol for Neuro-2A Cell

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, dilute one part of the stock solution with 4 parts of ddH_2O into a sterile bottle. The working solution is table at 4 °C~RT for 12 months.

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 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 siRNA transfection per cell culture vessel

Culture Dish	Growth Medium (ml)	Transfection Buffer (µL)	siRNA (pmoles) Final 30 nM	GenMute™ Reagent (µL)
24-well	0.5	50	15	1.5
12-well	0.75	75	23	2.5
6-well	1.0	100	30	3.0
60 mm	3.0	300	90	9.0
10 cm / Flask 75	8.0	800	240	25

Step III. siRNA Transfection Protocol:

For optimal siRNA-mediated silencing, we recommend using 30 nM siRNA. 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 minutes before transfection.
- Dilute 30 pmoles siRNA (final concentration of 30 nM respectively per well) into 100 µl of working solution of GenMute™ Transfection Buffer prepared in **Step I**. Pipette up and down to mix.

  Note: For maximum gene silencing, dilute siRNA and GenMute™ reagent with GenMute™ Transfection Buffer (1x).
  We strongly suggest reconstituting siRNA stock solution at 10 µM, so add 3.0 µl siRNA stock solution per well of 6-well plate to make final 30 nM siRNA.
- Add 3.0 µl GenMute™ reagent, mix by pipetting up and down.
- Incubate for ~15 minutes at RT to let transfection complex form.

  Note: Never keep the complex longer than 30 minutes.
- Add the transfection mix to the cells drop wise. Gently rock the plate back and forth and return the plate to CO_2 incubator.
- Replace transfection medium by cell growth medium ~5 hours after transfection when necessary.
- Gene silencing is usually measured 24~48 hours post transfection.

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