

# GenMute™ siRNA Transfection Reagent for Primary Neurons



10075 Tyler Place, Suite 19  
Ijamsville, MD 21754  
FAX. 301-560-4919  
TEL. 301-330-5966  
Toll Free. 1-(866)-918-6812  
Email: [info@signagen.com](mailto:info@signagen.com)  
Web: [www.signagen.com](http://www.signagen.com)

----- A General Protocol for Transfecting  
siRNA to Primary Neurons

- 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 the most powerful siRNA delivery tool. GenMute™ siRNA Transfection Reagent for primary neurons is pre-optimized for transfecting siRNA to primary neurons 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 primary neurons is being given below, optimization is sometimes needed for different siRNAs.

## Standard siRNA Transfection of Primary Neurons

### 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<sub>2</sub>O into a sterile bottle. The working solution is stable at 4 °C–RT for 12 months.

### Step II. Primary Neurons Preparation:

Primary neurons should be prepared to mix with 5 % astrocytes and glia cells per the standard procedures. Perform transfection is 4–7 days after plating.

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	2.0
6-well	1.0	100	30	3.0
60 mm	3.0	300	90	9.0
10 cm / Flask 75	8.0	800	240	20

### Step III. siRNA Transfection Protocol:

For optimal siRNA-mediated silencing, we recommend using 25 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 culture medium 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 15 µM, so add 2.0 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<sub>2</sub> incubator.
- Replace transfection medium by cell growth medium ~5 hours after transfection.
- 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