Cat # SL100568-KER Store at 4 °C

GenMute™ siRNA Transfection Reagent for Primary Keratinocytes

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

- ☐ 100 µl
- ☐ 500 µl
- ☐ 1000 µl

SignaGen® Laboratories
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
Web: www.signagen.com

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 keratinocytes is pre-optimized for transfecting siRNA to primary keratinocytes with maximum silencing.

Important Guidelines for Transfection:

- This reagent can be used for transfecting both primary and immortalized keratinocytes.
- 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 keratinocytes is being given below, optimization is sometimes needed for different siRNAs.

Standard siRNA Transfection Protocol for Primary Keratinocytes

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 ^{0}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 ~60% 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 40 nM	GenMute™ Reagent (µL)
24-well	0.5	50	20	1.2
12-well	0.75	75	30	2.0
6-well	1.0	100	40	2.4
60 mm	3.0	300	120	7.2
10 cm / Flask 75	8.0	800	320	20

Step III. siRNA Transfection Protocol:

For optimal siRNA-mediated silencing, we recommend using 40 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 40 pmoles siRNA (final concentration of 40 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 20 µM, so add 2.0 µl siRNA stock solution per well of 6-well plate to make final 40 nM siRNA.
- Add 2.4 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 ^{0}C. This item shipped at ambient temperature