

GenMute™ siRNA Transfection Reagent for Primary Keratinocytes

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

- ☐ 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 transfection reagent designed for highly efficient delivery of both siRNA and DNA. Utilizing our proprietary pH-Dependent Conformational Change (PDCC) technology, the biodegradable polymer has been chemically modified with pre-screened hydrophobic side groups to enhance cellular uptake and intracellular release. GenMute™ siRNA Transfection Reagent for primary keratinocytes pre-optimized for transfecting siRNA to primary keratinocytes with maximum silencing and minimal cytotoxicity.

Important Guidelines for Transfection

- For optimal transfection efficiency and gene silencing, always use GenMute™ Transfection Buffer to dilute both the siRNA/DNA and GenMute™ Reagent.
- The standard protocol below has been optimized for siRNA transfection into the primary keratinocytes. However, minor optimization may be required for different cell types or siRNA sequences.

Standard siRNA Transfection Protocol for the Primary Keratinocytes

Step I. Preparation of GenMute™ Transfection Buffer (1× Working Solution)

GenMute™ Transfection Buffer is supplied as a 5× concentrated stock solution. To prepare a 1× working solution, dilute one part of the 5× stock with four parts sterile ddH₂O in a sterile container. The resulting 1× working solution is stable for up to 24 months when stored at 4°C to room temperature (RT).

Step II. Cell Seeding

Seed cells 18–24 hours prior to transfection so that they reach approximately 60% confluency at the time of transfection. Add fresh complete culture medium containing serum and antibiotics to each well 30–60 minutes before transfection.

Note: GenMute™ Reagent is fully compatible with both serum and antibiotics. Therefore, culture medium containing serum and antibiotics may be used throughout the entire transfection experiment without compromising transfection efficiency.

Table 1. General Guidelines for siRNA Transfection According to Cell Culture Vessel Format

Culture Dish	Growth Medium (ml)	Transfection Buffer (µL)	siRNA (pmol) Final 40 nM	GenMute™ Reagent (µL)
24-well	1.0	50	40	2.0
12-well	1.5	75	60	3.0
6-well	2.0	100	80	4.0
60 mm	5.0	300	200	12
10 cm / Flask 75	10	600	400	24

Step III. siRNA Transfection Protocol

For optimal gene silencing, we recommend a final siRNA concentration of 40 nM. The protocol below is optimized for one well of a 6-well plate. For other culture vessel formats, please refer to Table 1.

- Add 2.0 mL of fresh complete culture medium containing serum and antibiotics to each well 30–60 minutes prior to transfection.
- Dilute 80 pmol siRNA (final concentration: 40 nM) into 100 µL of GenMute™ Transfection Buffer (1×) prepared in **Step I**. Mix gently by pipetting up and down.

Note: For maximum transfection efficiency and gene silencing, both the siRNA and GenMute™ Reagent should be diluted in GenMute™ Transfection Buffer (1×). We strongly recommend preparing the siRNA stock solution at 40 µM. Using this stock concentration, add 2.0 µL of siRNA stock per well of a 6-well plate to achieve a final siRNA concentration of 40 nM.

- Add 4.0 µL of GenMute™ Reagent to the diluted siRNA solution and mix gently by pipetting up and down.
- Incubate the mixture at room temperature (RT) for approximately 15 minutes to allow transfection complexes to form.

Note: Do not incubate the transfection complex for longer than 30 minutes.

- Add the transfection complex to the cells dropwise. Gently rock the plate back and forth to ensure even distribution and return the cells to a 37°C, 5% CO₂ incubator.
- If necessary, replace the transfection medium with fresh growth medium approximately 18 hours after transfection.
- Gene silencing can typically be evaluated 24–72 hours post-transfection.

Storage: GenMute™ siRNA Transfection Reagent is stable for up to 24 months at 4 °C. This item shipped at ambient temperature