

PepMute™ siRNA & DNA Transfection Reagent

----- A General Protocol for Transfecting
DNA to Mammalian Cells

- ☐ 100 µL
- ☐ 500 µL
- ☐ 1000 µL



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This product is for laboratory research ONLY and not for diagnostic use

Introduction:

PepMute™ Reagent is a novel biodegradable polymer-based transfection reagent designed for efficient delivery of siRNA and plasmid DNA. Utilizing our proprietary pH-Dependent Conformational Change (PDCC) technology, the biodegradable polymer has been chemically modified through incorporation of pre-screened hydrophobic moieties onto its side chains, making PepMute™ Reagent a versatile and highly effective gene delivery tool. PepMute™ Reagent has been validated for efficient and reproducible transfection of siRNA, plasmid DNA, and DNA/siRNA co-transfection across a broad range of mammalian cell types.

Important Guidelines for Transfection:

- PepMute™ Reagent was developed as a versatile gene delivery tool. While this protocol describes procedures for plasmid DNA transfection in mammalian cells, protocols for siRNA transfection or DNA/siRNA co-transfection are available on our website.
- For maximum transfection efficiency, we recommend using PepMute™ Transfection Buffer to dilute both DNA and PepMute™ Reagent. Alternatively, serum-free PBS can be used as a diluent. Do not use serum-free DMEM containing sodium pyruvate or Opti-MEM, which contains proteins.

Procedures for Transfecting DNA into Mammalian Cells

I. Preparation of Working Solution of PepMute™ Transfection Buffer:

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

II. Cell Seeding:

Cells should be seeded 18–24 hours prior to transfection so that the culture reaches approximately 80% confluency at the time of transfection. Fresh complete growth medium containing serum and antibiotics should be added to each well 30–60 minutes before transfection.

Table 1. Guidelines for DNA Transfection per Cell Culture Vessel

Culture Dish	Growth Medium (ml)	Transfection Buffer (1×) (µL)	Plasmid DNA (µg)	PepMute™ Reagent (µL)
24-well	1.0	50	0.5	1.5
12-well	1.5	75	0.75	2.25
6-well	2.0	100	1.0	3
60 mm	5.0	300	3.0	9
10 cm/flask 75	10	600	6.0	18

Note: PepMute™ Reagent is fully compatible with serum- and antibiotic-containing media; therefore, complete growth medium containing serum and antibiotics may be used throughout the entire transfection procedure.

III. DNA Transfection Protocol:

For plasmid DNA transfection experiments, we recommend using 1.0–2.0 µg DNA per well of a 6-well plate. As a starting condition, we recommend using 1.0 µg DNA and 3.0 µL PepMute™ Reagent per well of a 6-well plate, which typically provides satisfactory transfection efficiency with minimal cytotoxicity. The following protocol is described on a per-well basis for a 6-well plate. For other culture vessel formats, refer to **Table 1**.

- Add 2.0 mL of fresh complete growth medium containing serum and antibiotics to each well 30–60 minutes prior to transfection.
- Dilute 1.0 µg plasmid DNA into 100 µL of PepMute™ Transfection Buffer (1×). Mix gently by pipetting up and down.
- Add 3.0 µL PepMute™ Reagent and vortex briefly to mix.
- Incubate the mixture at room temperature for approximately 10 minutes to allow transfection complex formation. Do not incubate the complexes for longer than 20 minutes.
- Add the entire transfection complexes dropwise to the cells.
- Gently rock the plate back and forth to ensure even distribution and return the plate to the incubator.
- Replace the transfection medium with fresh complete growth medium approximately 24 hours post-transfection and analyze gene expression as required.

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