

PepMute™ Plus siRNA & DNA Transfection Reagent

----- A General protocol for transfecting 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™ Plus Reagent is an advanced version of PepMute™ siRNA Transfection Reagent. Through incorporation of several pre-screened hydrophobic moieties into its peptide backbone, PepMute™ Plus Reagent gains enhanced self-assembly capability upon binding nucleic acids, making it a versatile and highly efficient gene delivery tool. PepMute™ Plus Reagent has been validated for effective and reproducible transfection of siRNA as well as DNA/siRNA co-transfection across a broad range of mammalian cell types.

Important Guidelines for Transfection:

- PepMute™ Plus Reagent was developed as a highly efficient siRNA delivery reagent. For most adherent cell lines and primary cells, a final siRNA concentration of approximately 5 nM is generally sufficient to achieve up to 90% gene silencing. For difficult-to-transfect cell types, we recommend using a final siRNA concentration of approximately 50 nM.
- Although standard protocols for siRNA transfection and DNA/siRNA co-transfection are provided below, optimization of transfection conditions may be required to achieve maximal gene silencing efficiency depending on the cell type and experimental application.

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™ Plus Reagent (µL)
24-well	1.0	100	1.0	2.0
12-well	1.5	150	1.5	3.0
6-well	2.0	200	2.0	6.0
60 mm	5.0	500	5.0	10
10 cm/flask 75	10	1000	10	20

Note: PepMute™ Plus 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™ Plus 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 2.0 µg plasmid DNA into 200 µL of PepMute™ Transfection Buffer (1×). Mix gently by pipetting up and down.
- Add 6.0 µL PepMute™ Plus 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™ Plus siRNA & DNA Transfection Reagent is stable for up to 24 months at 4 °C. This item shipped at ambient temperature