Cat # SL100571 Store at 4 °C

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 upgraded version from PepMute™ siRNA transfection reagent. With addition of several pre-screened hydrophobic groups to its peptide backbone, PepMute™ Plus Reagent gains self-assembly capacity when binding nucleic acids, making PepMute™ Plus Reagent a versatile and most powerful gene delivery tool. PepMute™ Plus Reagent have been validated to effectively and reproducibly transfect single siRNA, DNA or co-transfect DNA/siRNA to variety of mammalian cells.

Important Guidelines for Transfection:

- PepMute™ Plus reagent was formulated as a versatile gene delivery tool. While this protocol gives procedures for DNA transfection, the protocol for siRNA transfection and DNA/siRNADNA co-transfection can be obtained from our website.
- For maximum gene silencing, using PepMute™ Transfection Buffer is a must.

Procedures fro Transfecting DNA to Mammalian Cells

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

PepMute™ 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 70~80% 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: PepMute™ Plus 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 DNA Transfection Per Cell Culture Vessel

Culture Dish	Growth Medium (ml)	Transfection Buffer (µL)	Plasmid DNA (µg)	PepMute™ Reagent (µL)
24-well	0.5	50	0.5	1.5
12-well	0.75	75	0.75	2.25
6-well	1.0	100	1.0	3
60 mm	3.0	300	3.0	9
10 cm/flask 75	8.0	800	8.0	24

Step III. DNA Transfection protocol:

For DNA transfection experiment, we recommend using 0.5~1.0 µg DNA per well in a 6-well plate. As a starting point, we recommend using 1.0 µg DNA and 3.0 µl PepMute™ reagent per well of a 6-well plate which usually give satisfactory efficiency without visible toxicity. The following conditions are given per well of 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 1.0 µg DNA into 100 µl of PepMute™ Transfection Buffer. mix by pipetting up and down.
- Add 3 µl PepMute™ reagent, pipette up and down to mix.
- Incubate for 10~15 min at RT to let transfection complex form. Never keep the complex longer than 20 minutes.
- Add the transfection complex to the cells drop wise.
- Gently rock the plate back and forth and return the plate to the incubator.
- Replace transfection medium by cell growth medium 5 hours after transfection when necessary and analyze as required.

Storage: PepMute™ Plus siRNA & DNA Transfection Reagent is stable for up to 12 months at 4 ^{0}C. This item shipped at ambient temperature

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