Cat # SL100571 Store at 4 °C

PepMute™ Plus siRNA Transfection Reagent

----- A Standard Protocol for siRNA Transfection of 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, And co-transfect DNA/siRNA to variety of mammalian cells.

Important Guidelines for Transfection:

- PepMute™ Plus reagent was formulated as a powerful siRNA delivery tool. For most adherent cell lines and primary cells, siRNA at ~5.0 nM is basically sufficient to obtain up to 90% gene silencing. For hard-to-transfect cells, we recommend using a final siRNA concentration of 50 nM.
- While the standard protocols for siRNA transfection and siRNA/DNA co-transfection are being given below, optimization is often needed for maximal gene silencing.

PART I. Standard siRNA Transfection of Adherent 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 ~50% 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 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 5.0 or **50** nM	PepMute™ Plus (µL)
24-well	0.5	50	2.5 / **25**	1.2 ~ **2.0**
12-well	0.75	75	3.75 / **38**	2.0 ~ **3.3**
6-well	1.0	100	5.0 / **50**	2.4 ~ **4.0**
60 mm	3.0	300	15 / **150**	7.2 ~ **12**
10 cm / Flask 75	8.0	800	40 / **400**	20 ~ **33**

Step III. siRNA Transfection Protocol:

For optimal siRNA-mediated silencing, we recommend using 1~100 nM siRNA. As a starting point, we recommend using 5.0 nM siRNA which usually gives satisfactory silencing result for most adherent cell lines or primary cells. For hard-to-transfection cells, we recommend using a final siRNA concentration of 50 nM. (bold & underlined in **Table 1**).

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 5.0 or **50** pmoles siRNA (final concentration of 5.0 or **50** nM respectively per well) into 100 µl of working solution of PepMute™ Transfection Buffer prepared in **Step I**. Pipette up and down to mix.

  Note: For maximum gene silencing, dilute siRNA and PepMute™ Plus reagent with PepMute™ Transfection Buffer (1x). We strongly suggest reconstituting siRNA stock solution at 10.0 µM, so add 0.5 or 5.0 µl siRNA stock solution per well of 6-well plate to make final 5.0 and 50 nM siRNA respectively.
- Add 2.4 µl or 4.0 µl (for hard-to-transfect cells, bold and underlined in **Table 1**) PepMute™ Plus 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.
- Gene silencing is usually measured 24~78 hours post transfection.

PART II. A Standard Protocol for DNA/siRNA Co-transfection

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.

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- ☐ 100 µl
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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% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 30~60 min before transfection.

Note: PepMute™ Plus reagent is NOT interfered by serum and antibiotics, therefore serum and antibiotic containing medium can be used during the entire experiment.

Table 2. A Guideline for DNA & siRNA Co-transfection Per Cell Culture Vessel

Culture Dish	Growth Medium (ml)	Transfection Buffer (µL)	Plasmid DNA (µg)	siRNA (pmoles) Final 5.0 nM	PepMute™ Plus (µL)
24-well	0.5	50	0.25	2.5	1.5
12-well	0.75	75	0.375	3.25	2.25
6-well	1.0	100	0.5	5.0	3
60 mm	3.0	300	1.5	15	9
10 cm / flask 75	8.0	800	4.0	40	24

Step III. DNA & siRNA co-transfection protocol:

For DNA/siRNA co-transfection experiment, we recommend using 0.25~0.5 µg DNA and 1~20 nM siRNA per well in a 6-well plate. As a starting point, we recommend using 0.5 µg DNA and 5.0 pmoles siRNA (final concentration 5.0 nM) per well of a 6-well plate which usually give satisfactory knockdown effect.

The following conditions are given per well of a 6 well plate. For other culture format, please refer to **Table 2**.

- For each well, add 1.0 ml of complete medium with serum and antibiotics freshly 30~60 minutes before transfection.
- Dilute 0.5 µg DNA and 5.0 pmoles siRNA (final 5.0 nM) into 100 µl of working solution of PepMute™ Transfection Buffer. Vortex to mix followed by brief spin to bring drops to the bottom of the tube.

  Note: For optimal transfection efficiency and maximum gene silencing, PepMute™ Transfection Buffer is a must for diluting siRNA/DNA and PepMute™ reagent. We strongly suggest preparing siRNA stock solution at 5.0 µM, so add 1.0 µl siRNA stock solution per well of 6-well plate to make final 5.0 nM of siRNA.
- Add 3 µl PepMute™ Plus reagent immediately, mix by pipetting up and down.
- Incubate for ~15 min at RT to let transfection complex form.
  Note: Never keep the complex longer than 30 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.
- Gene silencing is usually measured 24~72 hours post transfection.

Storage: PepMute™ Plus siRNA Transfection Reagent is stable for up to 12 months at 4 °C. This item shipped at ambient temperature