Cat # SL100566 Store at 4 °C

PepMute™ 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™ siRNA Transfection Reagent is a novel peptide based siRNA delivery tool which provides more than 95% silencing efficiency at 1 nM siRNA in variety of mammalian cells. With our proprietary peptide simulation technology (PST), PepMute™ reagent was identified and validated as an exceptionally efficient vector for condensing and transfecting short (under 100 bp) single or double stranded nucleic acids such as siRNA, miRNA mimics and DNA oligoes to wide spectrum of mammalian cells.

Important Guidelines for Transfection:

- PepMute™ reagent was formulated as a powerful siRNA delivery tool. For most adherent cell lines and primary cells, siRNA at ~5 nM is basically sufficient to obtain up to 90% gene silencing, as observed for Hela, MCF and NIH-3T3. For hard-to-transfect cells, we recommend using a final siRNA concentration of 30 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 (1x) is stable at RT for 24 months.

Note: Always keep PepMute™ Transfection Buffer (5x) at RT. If refrigerated, white precipitates may appear. It won't affect the transfection efficiency. After dilution with 4 parts of ddH_2O to make PepMute™ Transfection Buffer (1x) working solution, the white precipitates will disappear. Always keep PepMute™ Transfection Buffer working solution (1x) at RT.

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 min before transfection.

Note: PepMute™ 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 **30** nM	PepMute™ Reagent (µL)
24-well	0.5	50	2.5 / **15**	1.2 ~ **1.8**
12-well	0.75	75	3.75 / **23**	2.0 ~ **3.0**
6-well	1.0	100	5.0 / **30**	2.4 ~ **3.6**
60 mm	3.0	300	15 / **90**	7.2 ~ **10.8**
10 cm / Flask 75	8.0	800	40 / **240**	20 ~ **30**

Step III. siRNA Transfection Protocol:

For optimal siRNA-mediated silencing, we recommend using 1~50 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 30 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 min before transfection.
- Dilute 5.0 or **30** pmoles siRNA (final concentration of 5.0 or **30** 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™ reagent with PepMute™ Transfection Buffer working solution (1x).

  We strongly suggest reconstituting siRNA stock solution at 10 µM, so add 0.5 or 3.0 µL siRNA stock solution per well of 6-well plate to make final 5.0 and 30 nM siRNA respectively.
- Add 2.4 µL or **3.6** µL (for hard-to-transfect cells, bold and underlined in **Table 1**) PepMute™ reagent, mix by pipetting up and down.
- Incubate for ~15 min at RT to let transfection complex form.

  Note: Never keep the complex longer than 25 min.
- 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 24~72 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 (1x) is stable at RT for 24 months.

Note: Always keep PepMute™ Transfection Buffer (5x) at RT. If refrigerated, white precipitates may appear. It won't affect the transfection efficiency. After dilution with 4 parts

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☐ 100 µl
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of ddH_2O to make PepMute™ Transfection Buffer (1x) working solution, the white precipitates will disappear. Always keep PepMute™ Transfection Buffer working solution (1x) at RT.

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 60~70% 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™ 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™ Reagent (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 0.5~0.6 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 min 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 briefly to mix.

  Note: For optimal transfection efficiency and maximum gene silencing, PepMute™ Transfection Buffer (1x) is a must for diluting siRNA/DNA and PepMute™ reagent.

  We strongly suggest preparing siRNA stock solution at 10 µM, so add 0.5 µL siRNA stock solution per well of 6-well plate to make final 5.0 nM of siRNA.
- Add 3 µL PepMute™ 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 min.
- 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™ siRNA Transfection Reagent is stable for up to 12 months at 4 °C. Always keep PepMute™ Transfection Buffer (5x) at RT. This item is shipped at ambient temperature.