Cat # SL100566 Store at 4 °C

PepMute™ siRNA Transfection Reagent

----- A Reverse Transfection Protocol
for High Throughput Screening (HTS)

- ☐ 100 µl
- ☐ 500 µl
- ☐ 1000 µl

SignaGen® Laboratories
10075 Tyler Place, Suite 19
Ijamsville, MD 21754
FAX. 301-560-4919
TEL. 301-330-5966
Toll Free. 1-(866)-918-6812
Email: info@signagen.com
Web: www.signagen.com

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:

- For maximum gene silencing, PepMute™ Transfection Buffer is a must for diluting siRNA and PepMute™ reagent.
- While the standard transfection protocol for siRNA reverse transdection is being given below, optimization is often needed for maximal gene silencing.
- For standard protocols of siRNA transfection and siRNA/DNA co-transfection, please visit our website at www.signagen.com/pepmute

Reverse Transfection Protocol for High Throughput Screening (HTS).

In this procedure, siRNA / PepMute™ transfection mix is added or prepared in the wells and the cells are overlaid subsequently. This optimized protocol is a time-saving protocol where transfection and plating are performed on the same day. This procedure is suitable for automated experiments and particularly for High Throughput Screening (HTS) applications.

1. Preparation of the cells:

Trypsinize the cells and prepare a cell suspension in growth medium at the recommended cell density according to **Table 1**.

Table 1. Recommended number of cells for different cell culture vessels

Culture vessel	Cell Number per well	Volume per well	Cell number to prepare per plate
384-well	2500±500	50 µl	1000000±20000
96-well	7500±2500	125 µl	750000±250000
24-well	40000±10000	500 µl	625000±250000

2. Reverse transfection protocol:

The following protocol is given for transfection of siRNA duplexes at 2 nM per well in a 96-well plate. These conditions are provided as starting point for optimization of siRNA transfection. Refer to **Table 2** for transfection in other culture formats.

Table 2. Recommended conditions for siRNA transfection at 1 nM in various cell culture vessels

Culture vessel	siRNA / well (pmols)	Diluent / Well (µl)	PepMute™ / well (µl)
384-well	0.124	12 µl	0.25
96-well	0.3	25 µl	0.375
24-well	1.1	50 µl	0.75

- For transfection in 96 well-plate format, we recommend preparing a minimal volume of 100 µl transfection mix to ensure homogenous preparation of the complexes, then add 25 µl of the transfection mix per well.
- For 100 µl transfection mix, dilute 1.2 pmoles of siRNA duplexes into 100 µl of PepMute™ Transfection Buffer.
- Add 1.5 µl of PepMute™ reagent to the 100 µl of siRNA solution. Mix promptly by pipetting up and down.
- Add 25 µl transfection mix **immediately** per well and incubate at RT to allow transfection complexes to form.
  Note: Never keep transfection mix at RT longer than 20 minutes.
- Add 7500 cells per well (125 µl at 60 cells/µl) in complete culture medium onto the siRNA/PepMute™ complexes solution. The final volume per well is 150 µl and the siRNA concentration is 2.0 nM.
- Mix gently by rocking the plate back and forth and return the plate to CO_2 incubator at 37 °C.
- Gene silencing is usually measured between 24~72 h for mRNA levels and 48 to 96 h for proteins.

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