SignaGen® Laboratories *A Gene Delivery Company…*

PepMute™ Reagent Short Protocol – siRNA-DNA Co-transfection

DAY 1: Prepare PepMute™ Transfection Buffer (1x) working solution and cell seeding

► Dilute one part of stock solution of PepMute™ Transfection Buffer (5x) with 4 parts of ddH2O to make PepMute™ Transfection Buffer working solution (1x).

► Cell seeding = ~70% confluency at the day of transfection.

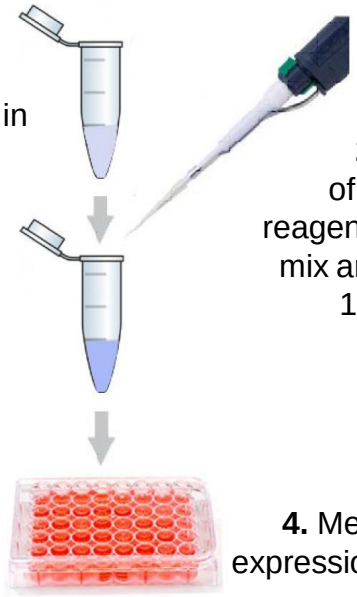
DAY 2: Transfection mix = PepMute™ reagent Z (µL) with X pmol siRNA at final 10 nM and Y µg DNA in W µL of transfection buffer (1x)

► Perform transfection in the presence of serum/antibiotics.

► Use PepMute™ Transfection Buffer (1x) only.

► Perform siRNA transfection referring to the picture and table below.

1. Dilute X pmol of siRNA & Y µg DNA in W µL of PepMute™ Buffer (1x). Vortex briefly to mix



2. Add Z µL of PepMute™ reagent. Vortex to mix and incubate 10 min at RT

3. Add transfection mix to the cells in serum- containing medium

4. Measure gene expression Kd 48 to 72 h post transfection

Culture Dish	Growth Medium (mL)	Transfection Buffer (1x) - W (µL)	Plasmid DNA - Y (µg)	siRNA X pmol Final 10 nM	PepMute Reagent (µL) - Z
24-well	1.0	50	0.25	10	1.5
12-well	1.5	75	0.375	15	2.25
6-well	2.0	100	0.5	20	3
60 mm	5.0	300	1.5	50	7.5
10 cm / flask 75	10	600	4.0	100	15

DAY 3~4: Measure knock-down of the gene expression

Visit the following link for complete protocol
https://signagen.com/products/PepMute-sirna-transfection-reagent