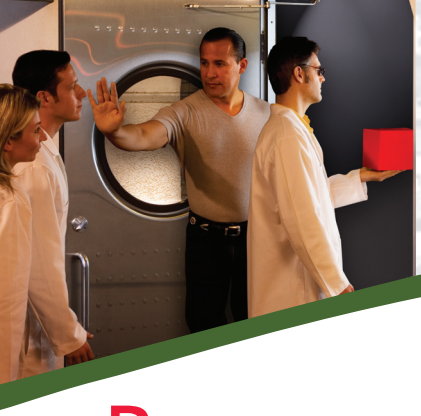

Reverse Transfection Using N-TER/siRNA Nanoparticles

Materials

- Tissue culture treated plates (96-well or other desired format)
- Serum-free medium
- 1x serum-supplemented medium
- 4x serum-supplemented medium
- Target siRNA(s)
- N-TER™ Nanoparticle siRNA Transfection System (Sigma, N2913)
- Water, Molecular Biology Reagent (Sigma, W4502)
- Sterile microcentrifuge tubes
- Microcentrifuge
- Vortex

For convenience, this protocol was modified to make enough siRNA complex for four replicate wells in a 96-well plate. The final volume of transfection medium is 0.1 mL per well with a final siRNA concentration of 40, 20, or 10 nM. If you wish to transfect with a different siRNA concentration, please refer to the "Scaling of the N-TER Nanoparticle Formation Reaction" section at the end of the document.

Preparation of the Nanoparticle Formation Solution

1. Thaw the N-TER Peptide (Sigma, N2788) and 5 µM siRNA working stock at room temperature for approximately 10 minutes. Briefly vortex each tube and pulse-spin in a microcentrifuge. Store the siRNA working stock on ice until it is needed.
2. Prepare the target siRNA dilution in a sterile 1.5 mL tube. Dilute the 5 µM target siRNA into the appropriate amount of siRNA Dilution Buffer (Sigma, N0413). Briefly vortex the tube and pulse-spin in a microcentrifuge. Store the diluted siRNA on ice until they are needed.

Reagent	siRNA Dilution	siRNA Mock
5 µM Target siRNA (µL)	6.5	0
siRNA Dilution Buffer (µL)	18.5	25
FINAL VOLUME (µL)	25	25

Table 1: Dilution of target and control siRNA into siRNA Dilution Buffer

3. Prepare the N-TER Peptide dilution in a sterile tube. Dilute the N-TER Peptide into the appropriate amount of water. Briefly vortex the tube and pulse-spin in a microcentrifuge.

Reagent	N-TER Dilution	N-TER Mock
N-TER Peptide (µL)	4	0
Water (µL)	21	25
FINAL VOLUME (µL)	25	25

Table 2: Dilution of N-TER Peptide into water

4. Prepare the target siRNA Nanoparticle Formation Solution (NFS) by adding the diluted siRNA to the diluted N-TER Peptide. Briefly vortex the tube and pulse-spin in a microcentrifuge.
5. Incubate the tube containing the NFS at room temperature for 20 to 30 minutes to allow the N-TER Peptide/siRNA nanoparticles to form.

NOTE: The concentration of the siRNA in the Nanoparticle Formation Solution is 650 nM at this point.

Dilution of the Nanoparticle Formation Solution (NFS)

Certain cell types do not transfect well in the presence of serum. For this reason, when performing N-TER transfections for the first time with a given cell type, we recommend performing the transfections both in the presence and absence of serum.

1. Set up eight sterile tubes.
2. Add serum-supplemented or serum-free medium to the appropriate tube as directed in Table 3. Then add the target siRNA and mock NFS to the appropriate tube. Make sure to mix thoroughly by inverting each tube several times. Then pulse-spin in a microcentrifuge.

Reagent	Serum-supplemented no pre-incubation				Serum-free pre-incubation			
	40 nM	20 nM	10 nM	Mock	40 nM	20 nM	10 nM	Mock
Target NFS (µL)	27	13.5	7	0	27	13.5	7	0
Mock NFS (µL)				27				27
Serum-supplemented medium (µL)	423	436.5	443	423	0	0	0	0
Serum-free medium (µL)	0	0	0	0	198	211.5	218	198
FINAL VOLUME (µL)	450	450	450	360	225	225	225	225

Table 3: Dilution of the target siRNA and mock NFS into serum-supplemented and serum-free medium

Reverse transfection in serum-supplemented or serum-free medium

1. Add 50 µL of each of each siRNA dilution in serum-supplemented transfection medium to the appropriate wells of a 96-well plate as indicated in Figure 1.
2. Add 25 µL of each of each siRNA dilution in serum-free transfection medium to the appropriate wells as indicated in Figure 1.

		Replicates			
Serum-supplemented	40 nM siRNA	1	2	3	4
	20 nM siRNA	1	2	3	4
	10 nM siRNA	1	2	3	4
	Mock Control	1	2	3	4
Serum-free	40 nM siRNA	1	2	3	4
	20 nM siRNA	1	2	3	4
	10 nM siRNA	1	2	3	4
	Mock Control	1	2	3	4

Figure 1: Transfection of cells in the presence and absence of serum

NOTE: Plates containing transfection medium can be stored covered at room temperature for several hours without negatively affecting transfection efficiency of the N-TER Peptide/siRNA complex.

3. Trypsinize adherent cells and resuspend in serum-supplemented medium or serum-free medium at 2x the desired final cell density.
4. Overlay 50 µL of cell suspension onto the NFS in the appropriate wells as indicated in Figure 1. Incubate the plate under standard cell culture conditions, typically 37 °C and 5% CO2, for 2-4°hours.
5. Add 25 µL of 4x serum-containing medium to each of the serum-free pre-incubation wells. Incubate at 37 °C and 5% CO2 overnight.
6. Replace the transfection medium with fresh serum-supplemented medium 24 hours post-transfection. Incubate at 37 °C and 5% CO_2 until they are ready to harvest.