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Product Information

N-TER™ Nanoparticle siRNA Transfection System

Catalog Number **N2913**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Traditional lipid-based siRNA transfection reagents exhibit a number of drawbacks, including a limited ability to transfect a variety of cell types, such as primary, neuronal, differentiated, and non-dividing cells. Sigma's N-TER™ Nanoparticle siRNA Transfection System is a peptide-based transfection reagent that is specifically designed to bypass these limitations, allowing for the efficient delivery of siRNAs into historically recalcitrant eukaryotic cell types.

The N-TER Peptide binds siRNAs non-covalently, forming a nanoparticle. The protocol for N-TER/siRNA nanoparticle formation is user-friendly and easily adapted for high-throughput applications. The resulting nanoparticle is generally very stable for at least 1 year at –20 °C (this may require some cell-type specific optimization). Additionally, N-TER/siRNA nanoparticles may be transfected into cells in the presence or absence of serum, allowing for the optimization of transfection conditions in a wide variety of cell types.

For optimal results, use fresh N-TER/siRNA complexes, please avoid multiple freeze-thaw cycles of N-TER/siRNA complexes as this may cause cell-type specific toxicity effects.

Reagents provided

- N-TER Peptide, Catalog Number N2788
- siRNA Dilution Buffer, Catalog Number N0413

Catalog Number	Package Size		
N2913	120 µL	400 µL	1 mL
Catalog Number	**Quantities Provided**		
N2788	120 µL	400 µL	1 mL
N0413	4 mL	14 mL	34 mL

Reagents and equipment required, but not provided

- Serum-free cell culture medium
- Complete cell culture medium (usually contains 10–15% serum)
- 2× serum medium (contains twice the amount of serum found in complete medium)
- Water, Molecular Biology Reagent, Catalog Number W4502
- Target siRNA(s)
- Positive and negative control siRNAs
- Sterile tissue culture-treated plates
- Microcentrifuge
- Sterile microcentrifuge tubes

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

If stored properly, the shelf life of this product is one year from the date of shipment. The N-TER Peptide should be stored at –20 °C. The N-TER Buffer may be stored at room temperature or at 2–8 °C.

Procedures

Preparation of the Nanoparticle Formation Solution

The detailed protocol is for the preparation of a standard 100 µL Nanoparticle Formation Solution (NFS). This provides sufficient NFS for the transfection of 4 wells of a 24-well plate with a final siRNA concentration of 20 nM in a volume of 0.6 mL per well. If you wish to transfect at a different siRNA concentration or into a different plate format, please refer to the "Scaling of the N-TER Nanoparticle Formation Reaction" section at the end of this document.

1. Thaw the N-TER Peptide and 5 µM siRNA working stocks at room temperature for ~10 minutes. Briefly vortex each tube and pulse-spin in a micro-centrifuge. Store the siRNA working stocks on ice until they are needed.
2. Prepare the **Target siRNA**, **Control (positive and/or negative) siRNA**, and **Cells only control** in sterile tubes (see Table 1). Add the appropriate amount of siRNA Dilution Buffer to Tubes 1A through 3A. Then, add the 5 µM target and control siRNA working stocks to Tubes 1A and 2A, respectively. Briefly vortex each tube and pulse-spin in a microcentrifuge. Store the diluted target and control siRNAs, and Cells only control on ice until they are needed.

Table 1.
Preparation of Target siRNA, Control siRNA, and Cells only control dilutions

Reagent	**Tube 1A** Target siRNA	**Tube 2A** Control siRNA	**Tube 3A** Cells only control
5 µM target siRNA	13 µL	0	0
5 µM control siRNA	0	13 µL	0
siRNA Dilution Buffer	37 µL	37 µL	50 µL
Final Volume	50 µL	50 µL	50 µL

3. Prepare the N-TER Peptide dilutions in sterile tubes (see Table 2). Add the appropriate amount of water to Tubes 1B through 3B. Then, add the N-TER Peptide to Tubes 1B and 2B. Briefly vortex each tube and pulse-spin in a microcentrifuge.

Table 2.
Preparation of N-TER Peptide dilutions

Reagent	**Tube 1B**	**Tube 2B**	**Tube 3B**
N-TER Peptide	8 µL	8 µL	0
Water	42 µL	42 µL	50 µL
Final Volume	50 µL	50 µL	50 µL

3. Prepare the **Target siRNA Nanoparticle Formation Solution (NFS)** by adding the contents of Tube 1A to Tube 1B (see Table 3). Briefly vortex each tube and pulse-spin in a microcentrifuge.
4. Add the contents of Tube 2A to Tube 2B and Tube 3A to Tube 3B to prepare **Control siRNA NFS** and the **Cells only control NFS**, respectively. Briefly vortex each tube and pulse-spin in a microcentrifuge.

Table 3.
Preparation of Target and Control Nanoparticle Formation Solutions

Reagent	**Tube 1B** Target SiRNA NFS	**Tube 2B** Control SiRNA NFS	**Tube 3B** Cells only control NFS
From Tubes 1A→3A	50 µL	50 µL	50 µL
From Tubes 1B→3B	50 µL	50 µL	50 µL
Final Volume	100 µL	100 µL	100 µL

Incubate the **Target siRNA NFS**, **Control siRNA NFS,** and the **Cells only control NFS** at room temperature for 15–20 minutes to allow the N-TER Peptide/siRNA nanoparticles to form in the NFS. Please note that the concentration of the siRNA in the NFS is 650 nM at this point.

Transfection of cells with the Nanoparticle Formation Solution

Depending on the cell type, the N-TER Peptide/siRNA nanoparticle may transfect the cells more efficiently in the presence or absence of serum (Methods 1 and 2, respectively). Sigma scientists have validated the two methods outlined in a number of cell types. It is recommended to transfect cells in parallel, both in the presence and absence of serum, the first time this procedure is performed.

For convenience, the volumes for four replicate wells in a 24-well plate are listed. The final volume of transfection medium is 0.6 mL per well with a final siRNA concentration of 20 nM. 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.

Suggested Cell Densities and Volumes for Transfection Assays

We recommend seeding cells at a density of 1.3×10^4 to 2.6×10^4 cells/cm^2, 16–20 hours prior to transfection. Use Table 4 to determine the recommended cell density and volume of medium that is recommended for your plate format.

Table 4.
Suggested cell densities and volumes for seeding cells to culture plates

Plate size	**Number of cells per well**	**Surface area per well** (cm^2)*	**Volume per Well** (mL)
96-well	$4.0–8.0 \times 10^3$	0.32	0.1
48-well	$1.2–2.4 \times 10^4$	0.95	0.3
24-well	$2.4–4.8 \times 10^4$	1.9	0.6
12-well	$4.8–9.6 \times 10^4$	3.8	1.2
6-well	$1.2–2.4 \times 10^5$	9.5	3.0

***** Surface areas listed are for Corning® Costar® tissue culture treated multiwell plates with flat-bottom wells. The dimensions of other manufacturers' plates may vary.

Method 1 – Serum-containing Transfection Medium

1. To make the **Target siRNA NFS** transfection medium, dilute 92 µL of the **Target siRNA NFS** into 2,908 µL of serum-containing medium and invert several times to mix.
2. Carefully remove the culture medium from each well, then transfer 600 µL of the **Target siRNA NFS** transfection medium to each of the four wells of the culture plate.
3. Repeat steps 1 and 2 with the diluted **Control siRNA NFS** and **Cells only control NFS**.
4. Incubate the plates under standard cell culture conditions, typically 37 °C and 5% CO_2, for 24 hours. If toxicity is a concern, the transfection medium can be removed in as little as four hours with similar levels of knockdown.
5. At 24 hours post-transfection, harvest the cells or exchange the transfection medium with fresh medium if you wish to grow the cells for a longer period of time.

Method 2 – Serum-free Transfection Medium

1. To make the **Target siRNA NFS** transfection medium, dilute 92 µL of **Target siRNA NFS** into 1,408 µL of serum-free medium and invert several times to mix.
2. Carefully remove the culture medium from each well, then transfer 300 µL of the **Target siRNA NFS** transfection medium to each of the four wells of the culture plate.
3. Repeat steps 1 and 2 with the diluted **Control siRNA NFS** and **Cells only control NFS**.
4. Incubate the plates at 37 °C and 5% CO_2 for 2–4 hours.
5. Add 300 µL of 2× serum medium to each well. If toxicity is a concern, the transfection medium can be removed and replaced with 600 µL of complete cell culture medium at this time.
6. Incubate the plates at 37 °C and 5% CO_2 for the remainder of the 24-hour incubation period.
7. At 24 hours post-transfection, harvest the cells or exchange the transfection medium with fresh medium if you wish to grow the cells for a longer period of time.

Scaling of the N-TER Nanoparticle Formation Reaction

Before beginning the N-TER nanoparticle transfection assay, consider a number of factors including: the size of the culture plates used, the range of siRNA concentrations to be screened, and the number of replicates performed. These factors will affect the amount of NFS prepared. Table 5 indicates the amount of NFS required for one well in a range of final siRNA concentrations and culture plate sizes. Please refer to Table 4 for suggested transfection medium volumes.

Use Table 5 to determine the amount of NFS required to transfect one well at the desired siRNA concentration(s). Then refer to Equation 1 to calculate the amount of NFS (NFS_{total}) needed for the assay. Using this equation, multiply the volume of NFS (from Table 5) by the number of wells to be transfected. Then multiply the result by 1.2 to ensure enough material for all of the replicates.

Please note the volumes of NFS in Table 5 are listed to the hundredth of a microliter. This is to aid in the accuracy of scaling calculations. Final calculations should be rounded to the nearest microliter.

Equation 1.

NFS_{total} = (volume of NFS/well) × number of wells × 1.2

Table 5.

Volume of Nanoparticle Formation Solution (NFS) to add per well for a range of siRNA concentrations**

Volume of NFS per well (µL)					
Final [siRNA] (nM)	**96 well plate**	**48 well plate**	**24 well plate**	**12 well plate**	**6 well plate**
50	7.69	23.08	46.15	92.31	230.77
40	6.15	18.46	36.92	73.85	184.62
30	4.62	13.85	27.69	55.38	138.46
20	3.08	9.23	18.46	36.92	92.31
10	1.54	4.62	9.23	18.46	46.15
5	0.77	2.31	4.62	9.23	23.08
1	0.15	0.46	0.92	1.85	4.61

** The concentration of the siRNA in the Nanoparticle Formation Solution prior to dilution in culture medium is 650 nM.

Unlike most lipid-based transfection reagents, the ratio of N-TER Peptide to target siRNA remains constant in the N-TER Nanoparticle siRNA Transfection System. When increasing the transfection volume or the number of transfections, the volumes of reagents used in preparing the NFS should be scaled accordingly. To scale volumes of reagents to be used in nanoparticle synthesis, multiply the volume of NFS_{total} calculated in Equation 1 by each of the numbers in Table 6. These values can then be substituted for the values listed in Tables 1 and 2.

Table 6.

Scaling Factors for Target siRNA, Control siRNA and N-TER Peptide dilutions

Reagent	**SiRNA** (µL)	**N-TER Peptide** (µL)
5 µM siRNA	0.13 × (NFS_{total})	–
N-TER Peptide	–	0.08 × (NFS_{total})
siRNA Dilution Buffer	0.37 × (NFS_{total})	–
Water	–	0.42 × (NFS_{total})
Final Volume	0.50 × (NFS_{total})	0.50 × (NFS_{total})

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