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ProductInformation

N-TER™ Nanoparticle siRNA Transfection System

Catalog Number **N0788**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The N-TER™ Nanoparticle siRNA Transfection System is a peptide-based system for use in the transient knockdown of eukaryotic gene expression.[1] The N-TER Peptide binds siRNAs non-covalently, forming a nanoparticle.[2] This nanoparticle interacts directly with lipids on the surface of the plasma membrane,[1,2] allowing the nanoparticle to diffuse across the cell membrane and deliver the siRNA directly to the cytoplasm. Unlike most lipid-based transfection methods, this novel delivery mechanism bypasses the endosomal pathway,[1,3,4] eliminating possible compartmentalization and degradation of the siRNA.[1,5]

N-TER nanoparticles have been experimentally validated in a wide variety of adherent cell lines, including primary cells, neuronal cells, differentiated cells, and non-dividing cells (see Table 1). The performance of N-TER nanoparticles has not been characterized with suspension cell lines.

Table 1.
Cell lines for which the N-TER Nanoparticle siRNA Transfection System has been experimentally validated

Cell Type	Source tissue
3T3-L1, differentiated adipocyte	mouse embryonic fibroblast cell line
A2780	human ovarian carcinoma cell line
A549	human lung carcinoma cell line
ASPC-1	human pancreatic carcinoma cell line
Astrocyte	human neuronal primary cell
Astrocytoma	human neuronal astrocytoma cell line
BSMC	human bronchial smooth muscle primary cell
C2C12, differentiated myocyte	mouse myoblastoma line
HeLa	human cervical adenocarcinoma cell line
HepG2	human hepatocarcinoma cell line
HT-29	human colorectal adenocarcinoma cell line
HUVEC	human umbilical vein epithelial primary cell
MCF7	human breast adenocarcinoma cell line
MDA-MB-231	human breast adenocarcinoma cell line
NHEK-AD	human adult keratinacyte primary cell
Raw264.7	mouse macrophage cell line
SW620	human colorectal adenocarcinoma cell line
U-87 MG	human brain glioblastoma cell line

Reagents

N-TER Peptide Catalog Number N0538	200 RXN
N-TER Buffer Catalog Number N0413	4 mL

The amounts provided are sufficient for up to 200 assays, assuming a final siRNA concentration of 30 nM in a final assay volume of 160 µL.

Reagents and Equipment Required, but Not Provided

- Serum-free cell culture medium
- Complete cell culture medium, usually contains 10% fetal bovine serum
- TE Buffer, Catalog Number T9285
- Water, Molecular Biology Reagent, Catalog Number W4502
- Hanks' Balanced Salt Solution, Catalog Number H6648, or Phosphate Buffered Saline, pH 7.4, Catalog Number P5493
- Target siRNA(s)
- Positive and negative siRNA controls
- Sterile 96 well cell culture plates, Catalog Number CLS3596
- Sterile microcentrifuge tubes
- 20-200 µL multichannel pipette
- Ultrasonic cleaner (sonicating water bath), with an operating frequency of 30-40 kHz
- Microcentrifuge

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, shelf life is 6 months from the date of shipment. The N-TER Peptide should be stored at –20 °C and the N-TER Buffer may be stored at room temperature.

User provided target siRNAs should be stored as concentrated master stocks (typically 20-50 µM) separated into small aliquots. The siRNA master stocks should be diluted to 5 µM working stocks in 1× TE Buffer or an equivalent siRNA dilution buffer.

Procedure

The following protocols are optimized for use in 96 well plates. Two transfection methods are provided. The Standard Method has been validated on all of the cells listed in Table 1. The Streamlined Method has been validated on only a subset of those cells to date, including HeLa and HepG2 cell lines, and astrocyte primary cells. However, it has several advantages over the Standard Protocol including fewer handling steps and the need for less transfection reagent and siRNA per assay.

When first testing a new target siRNA, screening a range of siRNA concentrations and cell densities is recommended to optimize two primary parameters:

1. knockdown of gene expression
2. cell viability

Optimized protocols for a select subset of cell types can be found at (www.sigma.com/nter). These protocols can eliminate the requirement for testing a broad range of cell densities. Additional protocols will be added to this site as they become available.

A suggested matrix for first-round screening of siRNAs in a 96 well plate format is provided in Appendix I. This matrix screens siRNA concentrations ranging from 30 nM to 2.5 nM and cell densities ranging from 5×10^3 cells per well to 2×10^4 cells per well. Additional screening may be used with narrower ranges to optimize transfection conditions. Enough N-TER Peptide/siRNA nanoparticle complex is prepared in each method to complete this matrix.

Standard Method Protocol

Preparation of adherent cells

Using the appropriate antibiotic-free complete medium, seed a sterile 96 well or alternate size culture plate 16-24 hours prior to transfection. Cell densities may need to be optimized for individual cell lines. For most cell types, optimal cell densities result in a confluence ranging from 30% to 70% on the day of transfection.

Formation of the N-TER Peptide/siRNA nanoparticle complex

1. Thaw the N-TER Peptide (Catalog Number N0538) and 5 µM siRNA working stocks at room temperature for ~10 minutes. Briefly vortex each tube and pulse-spin in a microcentrifuge. Store the siRNA working stocks on ice until they are needed.
2. Incubate the thawed N-TER Peptide in a sonicating water bath at maximum output and continuous power for 3-5 minutes.
   Note: Incubation of the N-TER Peptide in a sonicating water bath is optional. However, this step decreases possible aggregation of the peptide and can reduce the variability of transfection efficiency.

3. While the N-TER Peptide is in the sonicating water bath, dilute the 5 µM siRNA working stocks with N-TER Buffer (Catalog Number N0413) in a sterile tube (see Table 2). Briefly vortex each tube and pulse-spin in a microcentrifuge. Store the diluted siRNAs on ice until they are needed.

Table 2.
Dilution of 5 µM siRNA working stocks in N-TER Buffer

Reagent	**Tube 1A** Target siRNA	**Tube 2A** Neg. control siRNA	**Tube 3A** Cells only control
5 µM target siRNA working stock (µL)	39	0	0
5 µM negative control siRNA working stock (µL)	0	19.5	0
N-TER Buffer (µL)	111	55.5	75
FINAL VOLUME (µL)	150	75	75

4. Dilute the N-TER Peptide into water in a sterile tube (see Table 3). Briefly vortex each tube and pulse-spin in a microcentrifuge. Incubate the diluted N-TER Peptide in a sonicating water bath at maximum output and continuous power for 3-5 minutes.

Table 3.
Dilution of N-TER Peptide in water

Reagent	Tube 1B Target siRNA	Tube 2B Neg. control siRNA	Tube 3B Cells only control
N-TER Peptide (µL)	24	12	0
Water (µL)	126	63	75
FINAL VOLUME (µL)	150	75	75

5. Prepare the Nanoparticle Formation Solutions by combining the appropriate diluted siRNA solutions with diluted N-TER Peptide solutions by adding the contents of Tube 1A through 3A to Tube 1B through 3B, respectively (see Tables 2 and 3). Briefly vortex each tube and pulse-spin in a microcentrifuge.
6. Incubate the tubes containing the Nanoparticle Formation Solutions (combined siRNA and N-TER Peptide solutions) at 37 °C for 30-45 minutes to allow the nanoparticles to form.
   Note: The concentration of the siRNA in the Nanoparticle Formation Solution is 650 nM at this point.

Dilution of the N-TER Peptide/siRNA nanoparticles
The concentration of the siRNA in this method is based on a final experimental volume of 160 µL per well.

1. Combine equal volumes of water and N-TER Buffer to make 3 mL of 0.5× N-TER Buffer, which will be used to dilute the Nanoparticle Formation Solution to the appropriate concentrations. Vortex to mix and pulse-spin in a microcentrifuge.
2. Table 4 shows a dilution series for assay of a range of target siRNA concentrations. Dilute the 650 nM target Nanoparticle Formation Solution with 0.5× N-TER Buffer as indicated. Briefly vortex each tube and pulse-spin in a microcentrifuge.

Table 4.
Dilution of nanoparticles in 0.5× N-TER Buffer

	[siRNA]$_{final}$ (nM)				
Reagent	**30**	**20**	**10**	**5**	**2.5**
Nanoparticle Formation Solution (µL)	111	74	37	18	9
0.5× N-TER Buffer (µL)	339	376	413	432	441

3. Dilute the negative control Nanoparticle Formation Solution as indicated in the first column ([siRNA]$_{final}$ of 30 nM) of Table 4.
4. Dilute 14 µL of the target siRNA into 436 µL of 0.5× N-TER Buffer to use as the "siRNA only control." Briefly vortex each tube and pulse-spin in a microcentrifuge.
5. Dilute the 150 µL of Cells only control (Tube 3 in Tables 2 and 3) with 300 µL of 0.5x N-TER Buffer.

Note: Each of the above target siRNA and control dilutions will provide enough material for up to 15 replicates.

Transfection of adherent cells
1. Carefully remove the medium from each well, then wash each well with 100 µL of Hank's Balanced Salt Solution or PBS, pH 7.4. Be careful to avoid disturbing the cell layer at the bottom of the wells.
2. Transfer 30 µL of the diluted Nanoparticle Formation Solution ([siRNA]$_{final}$ of 30 nM) to each well of row A of the 96 well culture plate. Repeat this step in subsequent rows with the remaining diluted Nanoparticle Formation Solutions and controls as indicated in Appendix I until the culture plate is filled. Gently rock the plate to evenly distribute the liquid over the surface of the cells. Incubate the plate at room temperature for 3-5 minutes.
3. Carefully add 30 µL of serum-free medium to each well.
4. Repeat steps 1-3 for any additional culture plates.
5. Incubate the plates under standard cell culture conditions, typically 37 °C and 5% CO_2, for 2-4 hours.
6. Add 100 µL of complete medium to each well.
7. Incubate the plates under standard cell culture conditions, typically 37 °C and 5% CO_2, for 24-48 hours.

Streamlined Method Protocol

Preparation of adherent cells

Using the appropriate antibiotic-free complete medium, seed a sterile 96 well or alternate size culture plate 16-24 hours prior to transfection. Cell densities may need to be optimized for individual cell lines. For most cell types, optimal cell densities result in a confluence ranging from 30% to 70% on the day of transfection.

Formation of the N-TER Peptide/siRNA nanoparticle complex

1. Thaw the N-TER Peptide (Catalog Number N0538) and 5 µM siRNA working stocks at room temperature for ~10 minutes. Briefly vortex each tube and pulse-spin in a microcentrifuge. Store the siRNA working stocks on ice until they are needed.
2. Incubate the thawed N-TER Peptide in a sonicating water bath at maximum output and continuous power for 3-5 minutes.
   Note: Incubation of the N-TER Peptide in a sonicating water bath is optional. However, this step decreases possible aggregation of the peptide and can reduce the variability of transfection efficiency.
3. While the N-TER Peptide is in the sonicating water bath, dilute the siRNA working stocks into N-TER Buffer in a sterile tube (see Table 5). Briefly vortex each tube, and pulse-spin in a microcentrifuge. Store the diluted siRNAs on ice until they are needed.

Table 5.
Dilution of 5 µM siRNA working stocks in N-TER Buffer

Reagent	**Tube 1A** Target siRNA	**Tube 2A** Neg. control siRNA	**Tube 3A** Cells only control
5 µM target siRNA working stock (µL)	26	0	0
5 µM negative control siRNA working stock (µL)	0	13	0
N-TER Buffer (µL)	74	37	50
FINAL VOLUME (µL)	100	50	50

4. Dilute the N-TER Peptide into water in a sterile tube (see Table 6). Briefly vortex each tube and pulse-spin in a microcentrifuge. Incubate the diluted N-TER Peptide in a sonicating water bath at maximum output and continuous power for 3-5 minutes.

Table 6.
Dilution of N-TER Peptide in water

Reagent	**Tube 1B** Target siRNA	**Tube 2B** Neg. control siRNA	**Tube 3B** Cells only control
N-TER Peptide (µL)	16	8	0
Water (µL)	84	42	50
FINAL VOLUME (µL)	100	50	50

5. Prepare the Nanoparticle Formation Solutions by combining the appropriate diluted siRNA solutions with diluted N-TER Peptide solutions by adding the contents of Tube 1A through 3A to Tube 1B through 3B, respectively (see Tables 5 and 6). Briefly vortex each tube and pulse-spin in a microcentrifuge.
6. Incubate the tubes containing the Nanoparticle Formation Solutions (combined siRNA and N-TER Peptide solutions) at 37 °C for 30-45 minutes to allow the nanoparticles to form.
   Note: The concentration of the siRNA in the Nanoparticle Formation Solution is 650 nM at this point.

Dilution of the N-TER Peptide/siRNA nanoparticles

The concentration of the siRNA in this method is based on a final experimental volume of 100 µL per well.

1. Combine equal volumes of water and N-TER Buffer to make 1 mL of 0.5× N-TER Buffer, which will be used to dilute the Nanoparticle Formation Solution to the appropriate concentrations. Vortex to mix and pulse-spin in a microcentrifuge.
2. Table 7 shows a dilution series for assay of a range of target siRNA concentrations. Dilute the 650 nM Nanoparticle Formation Solution with 0.5× N-TER Buffer as indicated. Briefly vortex each tube and pulse-spin in a microcentrifuge.

Table 7.
Dilution of nanoparticles in 0.5× N-TER Buffer

	[siRNA]$_{final}$ (nM)				
Reagent	**30**	**20**	**10**	**5**	**2.5**
Nanoparticle Formation Solution (µL)	69	46	23	12	6
0.5× N-TER Buffer (µL)	81	104	127	138	144

3. Dilute the negative control as indicated in the first column ([siRNA]$_{final}$ of 30 nM) of Table 7.
4. Dilute 9 µL of the target siRNA into 141 µL of 0.5× N-TER Buffer to use as the "siRNA only control." Briefly vortex each tube and pulse-spin in a microcentrifuge.
5. Dilute the 150 µL of Cells only control (Tube 3 in Tables 5 and 6) with 300 µL of 0.5x N-TER Buffer.
6. Prepare a separate solution for each Nanoparticle Formation Solution dilution and control (steps 1 through 4) for incubation with the cells. Add 0.15 mL of a dilution or control to 1.35 mL of complete medium for a final volume of 1.5 mL. Invert 10 to 15 times to mix and pulse-spin in a microcentrifuge.

Note: Each of the above target siRNA and control dilutions will provide enough material for up to 15 replicates.

Transfection of adherent cells

1. Carefully remove the medium from each well. Then wash each well with 100 µL of Hank's Balanced Salt Solution or PBS, pH 7.4. Be careful to avoid disturbing the cell layer at the bottom of the wells.
2. Transfer 100 µL of the diluted Nanoparticle Formation Solution ([siRNA]$_{final}$ of 30 nM) in complete medium to each well of row A of the 96 well culture plate. Repeat this step in subsequent rows with the remaining Nanoparticle Formation Solution dilutions and controls in complete medium as indicated in Appendix I until the culture plate is filled.
3. Repeat steps 1-2 with for any additional culture plates.
4. Incubate the plates under standard cell culture conditions, typically 37 °C and 5% CO_2, for 24-48 hours.

Scaling of Reactions

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 number of transfections or the volume of transfections, the volumes of reagents used in preparing the Nanoparticle Formation Solution should be scaled accordingly. The volumes of reagents used in nanoparticle formation can be scaled down without adversely affecting the efficiency of nanoparticle formation. However, scaling the reaction up more than 2× may decrease the efficiency of complex formation. It is important that the ratio of N-TER Peptide to target siRNA be kept constant. Refer to Table 8 when calculating the volumes of the components required for the scaling of assays.

Table 8.
Ratios of components for the scaling of N-TER Peptide/siRNA nanoparticle formation reaction

Reagent	siRNA dilution	N-TER Peptide dilution
5 µM siRNA working stock (µL)	1.625	0
N-TER Peptide (µL)	0	1.000
N-TER Buffer (µL)	4.625	5.250
FINAL VOLUME (µL)	6.250	6.250

Table 9.
Volume of Nanoparticle Formation Solution required per well for a range of siRNA concentrations

	Volume of Nanoparticle Formation Solution required per well (µL)	
[siRNA]$_{final}$ (nM)	Standard Assay	Streamlined Assay
100	24.62	15.38
80	19.69	12.31
60	14.77	9.23
50	12.31	7.69
40	9.85	6.15
20	4.92	3.08
10	2.46	1.54
5	1.23	0.77

Table 9 provides information regarding the amount of Nanoparticle Formation Solution that is required per well for a range of siRNA concentrations. This table should be used to determine the amount of complex required for a given assay.

Downstream Procedures
Cell viability can be determined using standard laboratory procedures. Gene expression can be measured at the RNA or protein level. Steady-state levels of RNA transcripts can be assessed by quantitative RT-PCR or using a branched DNA assay such as the Quantigene® Reagent System. Protein expression may be quantified using antibody-based or chemical methods.

REFERENCES

1. Simeoni, F., *et al*., Insight into the mechanism of the peptide-based gene delivery system MPG: implications for delivery of siRNA into mammalian cells. *Nucleic Acids Res*., **31**, 2717-2724 (2003).
2. Morris, M.C., *et al*., A novel potent strategy for gene delivery using a single peptide vector as a carrier. *Nucleic Acids Res*., **27**, 3510-3517 (1999).
3. Friend, D.S., *et al*., Endocytosis and intracellular processing accompanying transfection mediated by cationic liposomes. *Biochim. Biophys. Acta*., **1278**, 41-50 (1996).
4. Xu, Y., and Szoka, F.C., Mechanism of DNA release from cationic liposome/DNA nanoparticles used in cell transfection. *Biochemistry*, **35**, 5616-5623 (1996).
5. Morris, M.C., *et al*., A new peptide vector for efficient delivery of oligonucleotides into mammalian cells. *Nucleic Acids Res*., **25**, 2730-2736 (1997).

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Appendix I
Suggested 96 well plate matrix for first round siRNA screening

			5K cells/well			10K cells/well			15K cells/well			20K cells/well		
			1	2	3	4	5	6	7	8	9	10	11	12
[Target siRNA]$_{final}$ (nM)	**30**	A	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
	20	B	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
	10	C	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**
	5	D	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**
	2.5	E	**49**	**50**	**51**	**52**	**53**	**54**	**55**	**56**	**57**	**58**	**59**	**60**
Negative siRNA control (30 nM)		F	**61**	**62**	**63**	**64**	**65**	**66**	**67**	**68**	**69**	**70**	**71**	**72**
siRNA only control (30 nM)		G	**73**	**74**	**75**	**76**	**77**	**78**	**79**	**80**	**81**	**82**	**83**	**84**
Cells only control		H	**85**	**86**	**87**	**88**	**89**	**90**	**91**	**92**	**93**	**94**	**95**	**96**