NeuroPORTER®

Transfection Reagent

Genlantis
A division of Gene Therapy Systems, Inc.

Catalog #	Content	Amount
T400101S	NeuroPORTER Transfection Reagent, Trial Size	0.2 ml
	DNA Diluent	1.0 ml
T400150 (75-300 rxns.)	NeuroPORTER ® Transfection Reagent, Dried Lipid Film	1 vial
	Hydration Buffer	1.5 ml
	DNA Diluent	7.5 ml
T400750 (375-1,500 rxns.)	NeuroPORTER ® Transfection Reagent, Dried Lipid Film	5 vials
	Hydration Buffer	5 x 1.5 ml
	DNA Diluent	5 x 7.5 ml

Shipping	Shipped at room temperature
Storage	Store kit at 4°C.

RELATED PRODUCTS	Catalog Numbers
GenePORTER® 3000 Transfection Reagent	T203007, 0.75 ml (107 reactions)
	T203015, 1.5 ml (214 reactions)
	T203115, 1.5 ml (2,140 reactions)
GenePORTER® Gold Transfection Reagent	T204015, 1.5 ml (400 reactions)
	T204030, 2 x 1.5 ml (800 reactions)
	T204115, 15 ml (4,000 reactions)
GenePORTER® 2 Transfection Reagent	T202007, 0.75 ml (75 reactions.)
	T202015, 1.5 ml (150 reactions.)
	T202075, 5 x 1.5 ml (750 reactions.)
GeneSilencer® siRNA Transfection Reagent	T500750, 0.75 ml (200 reactions.)
	T505750, 5 x 0.75 ml (1,000 reactions)
BioPORTER® Protein Delivery Reagent	BP502424, 24 single use tubes.
	BP509696, 96 single use tubes.
NeuroFect™ Transfection Reagent	T800075, 0.75 ml (75-300 reactions.)
	T800750, 5 x 0.75 ml (375-1500 rxns.)
MycoScope™ PCR Mycoplasma Detection Kit	MY01050 (50 reactions)
	MY01100 (100 reactions))

Introduction: NeuroPORTER® Transfection Reagent is a novel cationic lipid specially formulated for optimal transfection in neuronal cells, including primary neurons, differentiated post-mitotic neurons, neuronal cell lines, and glial cells. NeuroPORTER® Transfection Reagent is much easier to use than the traditional viral delivery method for transfecting DNA into neuronal cells. NeuroPORTER® Transfection Reagent is compatible with serum eliminates the need to change media following transfection. An included DNA Diluent is designed to facilitate DNA/lipid complex (lipoplex) formation and enhance the transformation efficiency in certain neuronal cells such as NT2 (not recommended for primary and differentiated neurons). Compared to other commercially-available transfection reagents, NeuroPORTER® provides superior transfection efficiency and minimized cytotoxicity. Cell type specific protocols are developed for NeuroPORTER® Transfection Reagents to ensure optimal transfection results.

Methods and Procedures

1. Transfection of Primary Rat Hippocampal Neurons

1.1. Seed primary rat hippocampal cells in poly-D-lysine coated plates (Becton Dickinson Labware) in the numbers listed in Table 1 below using the following Plating Medium: Neurobasal medium (Invitrogen Cat. No. 21103-049) supplemented with B27, 0.5 mM L-glutamine and 25 µM glutamate. Incubate the cells at 37ºC in 5% CO2 for 72 hours.

Table 1: Suggested Cell Plating Numbers.

Culture Vessel	Cell Number (per well)	Plating Medium Volume
96-well	15,000	0.125 ml
24-well	100,000	0.5 ml
12-well	200,000	1.0 ml
6-well	500,000	2.0 ml

1.2. After 72 hours of incubation, remove ½ volume of the Plating Medium and replace with the following Culture Medium: Neurobasal medium supplemented B27 and 0.5 mM L-glutamine (no 25 µM glutamate). Continue incubation for an additional 24 hours.

1.3. Hydrate the NeuroPORTER lipid vial at room temperature with 1.5 ml of the hydration buffer. Vortex for 30-60 seconds at top speed. Store the hydrated reagent at 4ºC and vortex briefly before use.

1.4. Dilute the DNA and hydrated NeuroPORTER reagent with serum-free medium. (do not use the DNA Diluent for primary neurons) Refer to Tables 2 and 3 for recommended DNA, NeuroPORTER, and serum-free medium volumes for different tissue culture plates.

Table 2: Volumes of Transfection Reagents.

DNA (µg)	Serum Free Medium for DNA (µl)	Neuro-PORTER (µl)	Serum Free Medium For Neuro-PORTER (µl)
0.1 – 0.5	12.5	2.5	10.0
1.0 – 3.0	25.0	5.0	20.0
2.0 – 4.0	37.5	7.5	30.0
4.0 – 6.0	62.5	12.5	50.0

1.5. Add the DNA solution to the diluted NeuroPORTER Transfection Reagent. Mix by pipetting up and down several times. Incubate at room temperature for 10 minutes to allow the NeuroPORTER /DNA complexes to form. Do not incubate for longer than 30 minutes.

Table 3: Medium Volumes and DNA Amount for Various Culture Dishes.

Culture Vessel	DNA (µg)	Serum Free Medium Volume (ml)	Total Transfection Volume (ml)
96-well	0.1-0.5	0.1	0.125
24-well	1.0-3.0	0.45	0.5
12-well	2.0-4.0	0.925	1.0
6-well	4.0-6.0	1.375	1.5

1.6. Remove the Plating Medium from the cells, and add the volume of serum-free medium indicated in Table 3 to each well.

1.7. Apply the DNA/NeuroPORTER complexes from step 1.5 to each well. The total transfection volume at this step is indicated in Table 3.

1.8. Gently mix the DNA/NeuroPORTER/serum-free medium by swirling, and place the cells in a 37ºC incubator with 5% CO2.

1.9. After two hours of incubation, add one additional volume of fresh Culture Medium containing 2X concentration of B27 onto the cells.

1.10. Perform assay for gene expression after 24-48 hours.

2. Transfection of Other Primary Neurons

2.1. Hydrate the NeuroPORTER lipid vial at room temperature with 1.5 ml of the hydration buffer. Vortex for 30-60 seconds at top speed. Store the hydrated reagent at 4ºC and vortex briefly before use.

2.2. Dilute the hydrated NeuroPORTER reagent with serum-free medium. Refer to Table 4 for the appropriate volume of serum-free medium

Table 4: Volumes of Transfection Reagents.

DNA (µg)	Serum Free Medium for DNA (µl)	Neuro-PORTER (µl)	Serum Free Medium for Neuro-PORTER (µl)
0.5	12.5	2.5	10.0
1.0	20.0	5.0	15.0
2.0	40.0	10.0	30.0
4.0	55.0	20.0	35.0
6.0	70.0	30.0	40.0
8.0	110.0	40.0	70.0

NOTE: *Although NeuroPORTER has been optimized for specific cell culture conditions, optimization may be needed to achieve maximum transfection efficiency. The two critical variables are the ratio of NeuroPORTER reagent to DNA and the quantity of DNA used. For optimization of the ratio of NeuroPORTER reagent to DNA start by using 2.5 to 15 µl of reagent for each 1 µg of DNA. Use a fixed amount of DNA or vary the amount as suggested in the Appendix to optimize this ratio.*

2.3. Dilute the DNA with the serum free medium (do not use the DNA Diluent for primary neurons). Refer to Table 4 for the appropriate volume of serum-free medium.

NOTE: *To obtain maximum efficiency in particular cells, some optimization may be needed. The two critical variables are the ratio of NeuroPORTER reagent to DNA and the quantity of DNA used. For optimization of the DNA quantity used, maintain a fixed ratio of NeuroPORTER reagent to DNA, and then vary the DNA quantity over a suggested range (see Table 5). See the Appendix for examples.*

2.4. Add the DNA solution to the diluted NeuroPORTER Transfection Reagent. Incubate at room temperature for 5 to 10 minutes to allow the NeuroPORTER /DNA complexes to form.

NOTE: *Do not incubate the DNA solution with the NeuroPORTER Transfection Reagent for longer than 30 minutes*

2.5. Add your complexes directly to the cells growing in serum-containing culture medium. Refer to Table 5 for suggested medium volumes.

Table 5: Medium Volumes and DNA Amount for Various Culture Dishes.

Culture Vessel	DNA (µg)	Plating Medium Volume (ml)
96-well	0.1-0.5	0.2
24-well	0.5-3.0	0.5
12-well	1.0-4.0	1.0
6-well	2.0-6.0	1.5
60 mm	6.0-8.0	2.5
100 mm	8.0-12.0	5.0

2.6. Add fresh growth media as needed 24 hours post transfection. Depending on the cell type and promoter activity, the assay for the reporter gene can be performed 24 to72 hours following transfection.

NOTE: *For some cell types, the old media can be replaced with fresh media at this step.*

3. Transfection of Neuronal Cell Lines

3.1. Hydrate NeuroPORTER lipid film at room temperature with 1.5 ml of the hydration buffer. Vortex for 30-60 seconds at top speed. Store the hydrated reagent at 4ºC and vortex briefly before use.

3.2. Dilute the hydrated NeuroPORTER reagent with serum-free medium. Refer to Table 6 for the appropriate volume of serum-free medium.

Table 6: Volumes of Transfection Reagents.

DNA (µg)	DNA Diluent (µl)	Neuro-PORTER (µl)	Serum Free Medium for NeuroPORTER (µl)
0.5	6.25	1.25	5.0
1.0	12.5	2.5	10.0
2.0	25.0	5.0	20.0
4.0	50.0	10.0	40.0

3.3. Dilute the DNA with the DNA Diluent and incubate 1 to 5 minutes at room temperature. Refer to Table 6 for the appropriate volume of DNA Diluent. Do not incubate DNA with the DNA Diluent for longer than 5 minutes. Avoid vortexing the DNA diluent.

NOTE: *Although NeuroPORTER consistently delivers high transfection efficiencies, in order to obtain maximum efficiency in particular cell types, some optimization may be needed. The two critical variables are the ratio of NeuroPORTER reagent to DNA and the quantity of DNA used. For optimization, first maintain a fixed ratio of NeuroPORTER reagent to DNA, and then vary the DNA quantity over the suggested range. If necessary, optimize the ratio of NeuroPORTER reagent to DNA by using 1.25 to 12.5 µl of reagent for each 1 µg of DNA. Use a low DNA quantity to optimize this ratio. Following this process, cell number can also be optimized. See the Appendix for examples.*

3.4. Add the DNA solution to the diluted NeuroPORTER Transfection Reagent. Incubate at room temperature for 5 to 10 minutes to allow the NeuroPORTER /DNA complexes to form. **Do not incubate the DNA solution with the NeuroPORTER Transfection Reagent for longer than 30 minutes.**

3.5. Add your complexes directly to the cells growing in serum-containing culture medium. Refer to Table 7 for suggested cell numbers for specific tissue culture dishes. Refer to Table 8 for appropriate medium volumes.

NOTE: *Cells plated the day before transfection should be 50% to 70% confluent on the day of transfection*

Table 7: Suggested Cell Culture Conditions for Transfection of Neuronal Cell Lines.

Culture Vessel	Number of Cells / Well
96-well	25.0-30.0 x 10^3
24-well	125.0-150.0 x 10^3
12-well	250.0-300.0 x 10^3
6-well	500.0-600.0 x 10^3
60 mm	1.0-1.5 x 10^6
100 mm	2.5-3.0 x 10^6

Table 8: Medium Volumes and DNA Amount for Various Culture Dishes.

Culture Vessel	DNA (µg)	Medium Volume (ml)
96-well	0.1-0.5	0.2
24-well	0.5-3	0.5
12-well	1.0-4.0	1.0
6-well	2.0-6.0	1.5
60 mm	6.0-8.0	2.5
100 mm	8.0-12.0	5.0

3.6. Add fresh growth media as needed 24 hours post transfection. Depending on the cell type and promoter activity, the assay for the reporter gene can be performed 24 to72 hours following transfection.

NOTES: *For some cell types, the old media can be replaced with fresh media at this step.*

The same protocol can be used to produce stably transfected cells: 48 to 72 hours post transfection, put the cells in fresh medium containing the appropriate selection antibiotic. It is important to wait at least 48 hours before exposing the transfected cells to the selection media. For some cell types it may be necessary to wait as long as 4 to 5 days before applying the selection condition.

4. Transfection of Differentiated Post-Mitotic Neurons and Glial Cell Lines

4.1. Hydrate NeuroPORTER lipid film at room temperature with 1.5 ml of the hydration buffer. Vortex for 30-60 seconds at top speed. Store the hydrated reagent at 4ºC and vortex briefly before use.

4.2. Dilute the hydrated NeuroPORTER reagent with serum-free medium. Refer to Table 9 for the appropriate volume of serum-free medium.

Table 9: Volumes of Transfection Reagents.

DNA (µg)	Serum Free Medium for DNA (µl)	Neuro-PORTER (µl)	Serum Free Medium For Neuro-PORTER (µl)
0.5	15.0	5.0	10.0
1.0	25.0	10.0	15.0
2.0	50.0	20.0	30.0
4.0	75.0	40.0	35.0
6.0	100.0	60.0	40.0
8.0	150.0	80.0	70.0

4.3. Dilute the DNA with the serum free medium. Refer to Table 9 for the appropriate volume of serum-free medium.

NOTE: *Although NeuroPORTER consistently delivers high transfection efficiencies, in order to obtain maximum efficiency in particular cell types, some optimization may be needed. The two critical variables are the ratio of NeuroPORTER reagent to DNA and the quantity of DNA used. For optimization, first maintain a fixed ratio of NeuroPORTER reagent to DNA, and then vary the DNA quantity over the suggested range. If necessary, optimize the ratio of NeuroPORTER reagent to DNA by using 5 to 20 µl of reagent for each 1 µg of DNA. Use a low DNA quantity to optimize this ratio. Following this process, cell numbers can also be optimized. See the Appendix for examples.*

4.4. Add the DNA solution to the diluted NeuroPORTER Transfection Reagent. Incubate at room temperature for 5 to 10 minutes to allow the NeuroPORTER /DNA complexes to form. Do not incubate the DNA solution with the NeuroPORTER Transfection Reagent for longer than 30 minutes.

4.5. Add your complexes directly to the cells growing in serum-containing culture medium. Refer to Table 10 for suggested cell number according to culture dishes size and cell types. Refer to Table 11 for appropriate medium volumes. Cells plated the day before transfection should be 50% to 70% confluent on the day of transfection.

Table 10: Suggested Cell Culture Conditions for Transfection of Differentiated Neurons and Glial Cells.

Culture Vessel	Cells / Well Diff. Neurons	Cells / Well Glial Cells
96-well	35×10^3	50×10^3
24-well	150×10^3	200×10^3
12-well	300×10^3	400×10^3
6-well	600×10^3	800×10^3
60 mm	1.5×10^6	2×10^6
100 mm	3×10^6	4×10^6

Table 11: Medium Volumes and DNA Amount for Various Culture Dishes.

Culture Vessel	DNA (µg)	Medium Volume (ml)
96-well	0.1-0.5	0.2
24-well	0.5-3.0	0.5
12-well	1.0-4.0	1.0
6-well	2.0-6.0	1.5
60 mm	6.0-8.0	2.5
100 mm	8.0-12.0	5.0

4.6. 24 hours post transfection, add fresh growth media as needed. Depending on the cell type and promoter activity, the assay for the reporter gene can be performed 24 to72 hours following transfection.

NOTE: *For some cell types, the old media can be replaced with fresh media at this step. Also, the same protocol can be used to produce stably transfected cells: 48 to 72 hours post transfection, put the cells in fresh medium containing the appropriate selection antibiotic. It is important to wait at least 48 hours before exposing the transfected cells to the selection media. For some cell types it may be necessary to wait as long as 4 to 5 days before applying the selection condition.*

APPENDIX – Transfection Optimization Examples

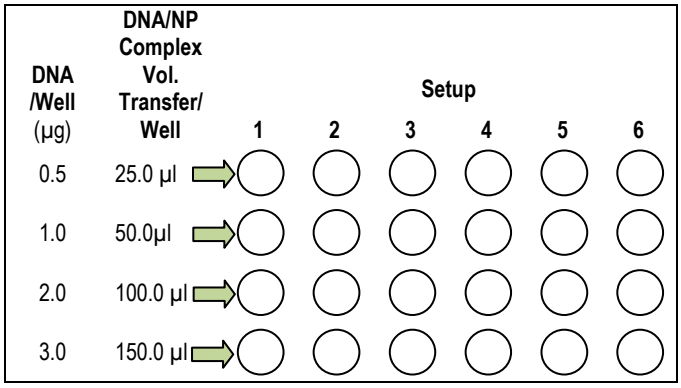
I. Optimization conditions for primary neuron transfection in 24-well plates

Follow the general protocol to prepare the DNA/NeuroPORTER complexes. We **do not recommend** using the DNA Diluent for primary neurons.

Setup	DNA dilutions in SFM*	NeuroPORTER dilutions in SFM*	Total Volume	Final DNA Concentration
1	10 µg in 250 µl	25 µl in 225 µl	500 µl	20 µg/ml
2		50 µl in 200 µl		
3		75 µl in 175µl		
4		100 µl in 150 µl		
5		125 µl in 125 µl		
6		150 µl in 100 µl		

***SFM = Serum-free medium**

Add the appropriate volume of complexes solution directly to your cells as illustrated below.



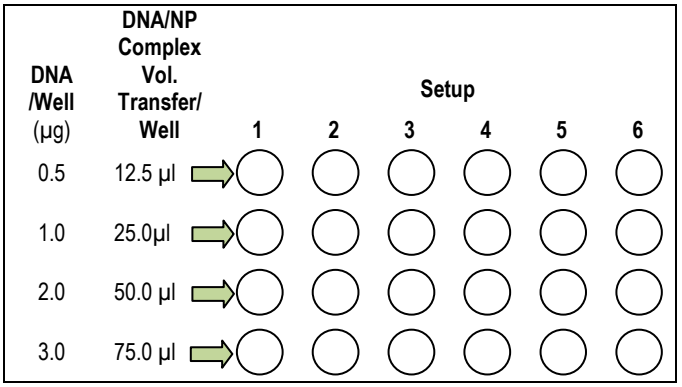
II. Optimization conditions for neuronal cell line transfection in 24-well plates

Follow the general protocol to prepare the DNA/NeuroPORTER complexes. We **recommend** using the DNA Diluent for neuronal cell lines such as NT2.

Setup	DNA dilutions in SFM*	NeuroPORTER dilutions in SFM*	Total Volume	Final DNA Concentration
1	10 µg in 125 µl	12.5 µl in 112.5 µl	250 µl	40 µg/ml
2		25 µl in 100 µl		
3		50 µl in 75µl		
4		75 µl in 50 µl		
5		100 µl in 25 µl		
6		125 µl NP Only		

***SFM = Serum-free medium**

Add the appropriate volume of complexes solution directly to your cells as illustrated below.

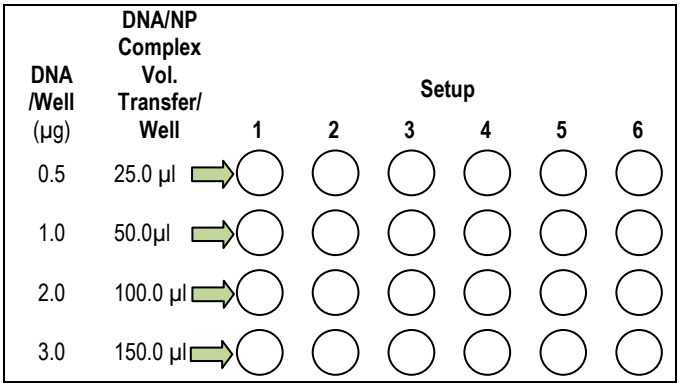


III. Optimization conditions for differentiated post-mitotic neurons and glial cell line transfection in 24-well plates

Follow the general protocol to prepare the DNA/NeuroPORTER complexes. We **do not recommend** using the DNA Diluent for differentiated post-mitotic neurons and glial cells.

Setup	DNA dilutions in SFM*	NeuroPORTER dilutions in SFM*	Total Volume	Final DNA Concentration
1	10 µg in 250 µl	50 µl in 200 µl	500 µl	20 µg/ml
2		75 µl in 175µl		
3		100 µl in 150 µl		
4		125 µl in 125 µl		
5		150 µl in 100 µl		
6		200 µl in 50 µl		

Add the appropriate volume of complexes solution directly to your cells as illustrated below.



Quality Control

To assure the performance of each lot of the NeuroPORTER reagent, we pre-qualify the chemical synthesis of NeuroPORTER lipid by mass spectrometry and thin layer chromatography. The final product is further tested by in vitro β-galactosidase transfection assay in NT2 neuronal precursor cell. Each lot shall have an acceptance specification of >70% of the activity of the Reference lot.