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ProductInformation

NeuroPORTER™ Transfection Kit

Product Code **NPT01**
Storage Temperature 2-8 °C

TECHNICAL BULLETIN

Product Description

The NeuroPORTER™ Transfection Kit is the latest innovation in DNA transfection for neuronal cells. Historically, cultured and primary neuronal cells have been extremely difficult to maintain and transfect. Transfection reagent sensitivity to media and serum, and cytotoxicity frequently resulted in low transfection efficiency, poor cell viability, and neurodegeneration. The advantages of the NeuroPORTER kit include excellent transfection efficiency, very low cytotoxicity, and no neurodegeneration or withdrawal of neurites, which is frequently observed when using calcium phosphate or electroporation. The NeuroPORTER system is compatible with serum, which means transfections may be performed in complete culture medium and eliminates the need to change the medium following transfection. NeuroPORTER Transfection Reagent is easier to use than traditional viral delivery for transfecting DNA into neuronal cells.

This kit contains the NeuroPORTER Transfection Reagent, a unique cationic lipid that has been specifically optimized for the transfection of neuronal cells. The reagent quickly forms a non-covalent complex with the DNA, creating a protective vehicle for immediate delivery into the cells. The optimized DNA Diluent is specifically recommended for cultured, non-differentiated cells such as NT2, but not recommended for use in primary cells. This diluent is designed to enhance transfection efficiency in cultured neuronal cell lines.

The NeuroPORTER Transfection Kit (Product Code NPT01) has sufficient reagents for 75 to 300 transfection reactions (2 µg of DNA per reaction) depending on cell type.

Components

NeuroPORTER Transfection Reagent, Product Code T 2823	1 vial
Hydration Buffer, Product Code H 9036	1.5 ml
DNA Diluent, Product Code D 1941	7.5 ml

Precautions and Disclaimer

The NeuroPORTER Transfection Kit and its components are sold for laboratory research use only. They are not for clinical diagnostic or human use. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

The NeuroPORTER Transfection Reagent (Product Code T 2823) appears as a dried lipid film. This film is reconstituted by adding 1.5 ml of the Hydration Buffer (Product Code H 9036) to the vial at room temperature. Vortex for 30 to 60 seconds at high speed. Store the reconstituted reagent at 2-8 °C. Vortex the Reconstituted NeuroPORTER Reagent briefly before each use.

Storage/Stability

The NeuroPORTER Transfection kit is shipped at ambient temperature and long term storage of all kit components at 2-8 °C is recommended.

Procedures

A. Transfection of Primary Neurons

Although NeuroPORTER has been optimized for specific cell conditions, some optimization may be necessary to achieve maximum transfection efficiency. The two critical variables are the ratio of Reconstituted NeuroPORTER Reagent to DNA and the quantity of DNA used. For optimization of the ratio of Reconstituted NeuroPORTER Reagent to DNA, the volume of Reconstituted NeuroPORTER Reagent may vary from 2.5 to 15 µl for each µg of DNA.

For optimization of the DNA quantity, maintain a fixed ratio of Reconstituted NeuroPORTER Reagent to DNA and vary the DNA quantity over the ranges suggested in Table 3.

1. Dilute the Reconstituted NeuroPORTER Reagent in the appropriate amount of serum-free medium (See Table 1).

   Table 1.
   Reconstituted Transfection Reagent to Medium Ratios (µl:µl)

Reconstituted NeuroPORTER Reagent (µl)	Serum-free Medium (µl)
2.5	10
5	15
10	30
20	35
30	40
40	70

2. Dilute the DNA in the appropriate volume of serum-free medium (see Table 2).
   Note: Do NOT use DNA Diluent for primary cells.

   Table 2.
   DNA to Medium Ratios (µg:µl)

DNA (µg)	Serum-free Medium (µl)
0.5	12.5
1	20
2	40
4	55
6	70
8	110

3. Add the DNA solution to the diluted NeuroPorter Transfection reagent. Incubate the mixture at room temperature for 5 to 10 minutes to allow the formation of DNA/NeuroPORTER complexes.
   Note: Do not incubate longer than 30 minutes.

4. Add the solution of DNA/NeuroPORTER complexes directly to the cells growing in complete medium containing serum. Refer to Table 3 for suggested medium volumes.

Table 3.
Complete Medium Volumes and DNA Amount for Various Culture Vessels

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

5. Add fresh growth medium as needed 24 hours post-transfection. The assay for reporter gene activity can be performed 24 to 72 hours following tranfection, depending on the nature of the cell type and nature of the reporter gene.

B. Transfection of Cultured Neuronal Cell Lines

Although NeuroPORTER delivers high transfection efficiencies, to obtain maximum efficiency in particular cells, some optimization may be necessary. The two critical variables are the ratio of Reconstituted NeuroPORTER Reagent to DNA and the quantity of DNA used. For optimization of the ratio of Reconstituted NeuroPORTER Reagent to DNA, the volume of Reconstituted NeuroPORTER Reagent may vary from 1.25 to 12.5 µl for each µg of DNA.

For optimization of the DNA quantity, maintain a fixed ratio of Reconstituted NeuroPORTER Reagent to DNA and vary the DNA quantity over the ranges suggested in Table 3.

1. Dilute the Reconstituted NeuroPORTER Reagent in the appropriate amount of serum-free medium (See Table 4).

   Table 4.
   Reconstituted Transfection Reagent to Medium Ratios (µl:µl)

Reconstituted NeuroPORTER Reagent (µl)	Serum-free Medium (µl)
1.25	5
2.5	10
5	20
10	40
15	60
20	80

2. Dilute the DNA in the appropriate volume of DNA Diluent, Product Code D 1941 (see Table 5) and incubate for 1 to 5 minutes at room temperature. Note: Do not incubate for longer than 5 minutes and avoid vortexing.

Table 5.
DNA to DNA Diluent Ratios (µg:µl)

DNA (µg)	DNA Diluent (µl)
0.5	6.25
1	12.5
2	25
4	50
6	75
8	100

3. Add the DNA solution to the diluted NeuroPorter Transfection reagent. Incubate the mixture at room temperature for 5 to 10 minutes to allow the formation of DNA/NeuroPORTER complexes. Note: Do not incubate longer than 30 minutes.

4. Add the solution of DNA/NeuroPORTER complexes directly to the cultured cells growing in complete medium containing serum. Refer to Table 3 for suggested medium volumes and Table 6 for suggested optimal cell density for specific culture vessels. Cells plated the day before transfection should be 50 to 70% confluent on the day of transfection.

Table 6.
Suggested Cell Density for Specific Culture Vessels.

Culture Vessel	Number Cells per Well
96 well plate	$2.5–3.0 \times 10^4$
24 well plate	$1.25–1.5 \times 10^5$
12 well plate	$2.5–3.0 \times 10^5$
6 well plate	$5.0–6.0 \times 10^5$
60 mm	$1.0–1.5 \times 10^6$
100 mm	$2.5–3.0 \times 10^6$

5. Add fresh growth medium as needed 24 hours post-transfection. The assay for reporter gene activity can be performed 24 to 72 hours following tranfection, depending on the nature of the cell type and nature of the reporter gene.
Note: The same protocol can be used to produce stably transfected cells, 48-72 hours post-transfection, place 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 medium. For some cell types it may be necessary to wait as long as 4 to 5 days before applying selections conditions.

Related Products

Product	**Product Code**
DME/F-12 - Sterile liquid medium with HEPES and sodium bicarbonate	D 6421
DME/F-12 - Sterile liquid medium with HEPES, sodium bicarbonate, and L-glutamine	D 8437
DME/F-12 - Sterile liquid medium with sodium bicarbonate and L-glutamine	D 8062
Fetal Bovine Serum, U.S.A. Origin, Sterile, Low Endotoxin	F 2442
Nerve Terminal Stain Kit I	NTS-I
Nerve Terminal Stain Kit II	NTS-II
Nerve Terminal Stain Kit V	NTS-V

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