NeuroTarget Transfection Agent

Catalog Number: NA2020

Kit Contents:

Component	Amount	Storage Condition
Rabies Viral Glycoprotein – 9R (1mg/mL) Molecular Weight 4,843.7g/mol	200 µL	-15°C to -25°C
Positive Control fluorescent miRNA (10µM/180ng/µl) Cy3	50 µL	-15°C to -25°C

Background:

NeuroTarget Transfection agent is composed of a cation-tagged rabies viral glycoprotein (RVG) that has been experimentally shown to deliver miRNA, siRNA and DNA inside neuronal cell lines containing the nicotinic acetylcholine receptor (nAChR). This peptide is highly efficient for targeted delivery of neuronal cells in vitro and in vivo.

This chimeric peptide is a fragment derived from rabies virus glycoprotein (RVG). Because neurotropic viruses cross the blood-brain barrier to infect brain cells, the same strategy may be used to enter the central nervous system and deliver siRNA to the brain. To enable siRNA binding, this chimeric peptide was synthesized by adding nonamer arginine residues at the carboxy terminus of RVG. This RVG-9R peptide was able to bind and transduce siRNA to neuronal cells in vitro, resulting in efficient gene silencing. After intravenous injection into mice, RVG-9R delivered siRNA to the neuronal cells, resulting in specific gene silencing within the brain. RVG-9R provides a safe and noninvasive approach for the delivery of siRNA and potentially other therapeutic molecules across the blood–brain barrier.

Required Materials not provided:

- Micro-pipettes
- RNase and DNase Free Disposable plastic tips
- Timer
- Cells and cell culture supplies (neuronal cells evaluated with this reagent in house TE671, Neuro-2a and SJCRH30 cell lines
- Fluorescent microscope or microtiter plate reader.

The NeuroTarget Transfection Reagent features:

- Compatibility with a broad range of neuronal cell types
- Simple, rapid protocol with fewer steps for consistent, reproducible results
- Easy optimization due to excellent cell viability
- No need to remove complexes from the cells
- Can be used with reverse or forward transfection method
- Superior adaptability to automated or robotic systems

Assay Protocol:

Scale Up and Optimization Ranges:

Culture Dish	Media Area	Final Volume	NeuroTarget	Dilute NeuroTarget To:	pmoL RNA	Cells Per Well
96-well	0.3 cm^2	100 µL	0.2-0.8 µL	10 µL	1-2 µL	0.4-0.6 x 10^4
24-well	2 cm^2	500 µL	0.6-2.4 µL	25 µL	5 – 10 µL	3-6 x 10^4
12-well	4 cm^2	1 mL	1-4 µL	50 µL	50 µL	7.5-15 x 10^4
6-well	10 cm^2	2 mL	2-8 µL	100 µL	100 µL	15-30 x 10^4

Use the procedure below to transfect RNA into eukaryotic cells in a **24-well format** (this is the most user-friendly scale for new users). Cells are first plated at specific cell densities in the wells of tissue culture plates within a laminar flow hood the day prior to performing the transfection experiment. On the day of the transfection, the transfection agent and RNA is prepared and added to the cells.

☝ The described amounts and volumes are on a per well basis.

1. **The day before transfection:** Plate between 5-10 x 10^4 cells in 500 µL of growth medium containing all your normal additives.

2. **The day of transfection:** Aspirate the cell growth medium, wash once with media lacking serum and replace with 450µL of fresh serum free medium.

3. **For each well, prepare *NeuroTarget* Transfection Reagent-RNA Complexes as follows:**
   a. Dilute 3µL *NeuroTarget RNA Transfection Reagent* in 50µL serum free medium
   b. Add 5-10 pmol siRNA and mix gently
   c. Incubate for 10-15 minutes at room temperature
   d. Overlay the *NeuroTarget* RNA Complexes (50µL) onto cells in each well
   e. Mix by gently tapping the sides of plate

4. After 6-12 hours at 37°C in a CO_2 incubator add 200µL of fresh growth media containing serum.

5. Incubate the cells at 37°C in a CO_2 incubator until ready to harvest. A typical siRNA knockdown reaction can take 48 to 72 hours, but longer or shorter period may be needed to see the impact of interest.

Transfection Optimization:

The most critical parameter for optimizing the transfection of RNAs is determining how much *NeuroTarget* to use with the matched number of cells, RNA and *NeuroTarget*. After determining the optimal transfection reagent volume, follow the fine-tuning method (see Fine-Tuning the Transfection Conditions) described below. The goal is to establish a balance between knockdown and cellular viability. To ensure reproducibility always keep the established conditions constant between experiments for a given cell type.

Quick reference:

Culture Vessel	Surface Area per Well	Volume of Plating Medium	NeuroTarget	RNA
96-well	0.3 cm2	100 µL	0.3 µL	3 pmol
48 well	0.6 cm2	250 µL	0.75 µL	7.5 pmol
24-well	2 cm2	500 µL	1.5 µL	15 pmol
12-well	4 cm2	1.0 mL	3 µL	30 pmol
6-well	10 cm2	2.0 mL	7.5 µL	75 pmol
60-mm	20 cm2	5 mL	17 µL	166 pmol
10cm	60 cm2	10 mL	43 µL	434 pmol
T 75	75 cm2	15 mL	59 µL	592 pmol
T 175	175 cm2	35 mL	138 µL	1382 pmol

Fine-tuning the Transfection Conditions:

Optimize cellular health by adjusting the time that cells are exposed to transfection complexes. Most cells don't require a media change during the transfection period; however, many cells will benefit from fresh media additions and the removal of the transfection complexes. Test your cells by replacing the transfection media after 8 or 24 hours.

User References:

- The average molecular weight (MW) of a siRNA is 13,300 g/mol.
- The average MW of a miRNA mimic is 14,100 g/mol.
- The average MW of a miRNA hairpin inhibitor is 18,500 g/mol

To convert between nmol to µg of siRNA?

- Multiply the number of moles by the MW for your oligo.
    - For example, 5 pmol of siRNA would be: (5 pmol) (13,300 pg/pmol) = 66.5 ng