# NeuroFECT™

## Neuronal Transfection Reagent

Genlantis
A division of Gene Therapy Systems, Inc.

### Contents

| Catalog # | Content | Amount |
|---|---|---|
| T800075 | NeuroFECT Transfection Reagent, 75 - 300 rxns | 0.75 ml |
| T800750 | NeuroFECT Transfection Reagent, 375 - 1500 rxns | 5 x 0.75 ml |

### Related Products

| Catalog # | Content | Amount |
|---|---|---|
| N100200 | NeuroPure™ E18 Primary Rat Hippocampal Cells | 1 x $10^6$ cells |
| N200200 | NeuroPure™ E18 Primary Rat Cortical Cells | 2 x $10^6$ cells |

### Related Products (Continued)

| Catalog # | Content | Amount |
|---|---|---|
| N300200 | NeuroPure™ P8 Rat Cerebellar Cells | 4 x$10^7$ cells |
| N400200 | NeuroPure™ E18 Primary Rat Hypothalamus | 1 pair |
| N500200 | NeuroPure™ E18 Primary Rat Striata | 1 pair |
| N600200 | NeuroPure™ E18 Primary Rat Spinal Cord | 1 spinal cord |
| N700200 | NeuroPure™ E18 Primary Rat Midbrain | 1 midbrain |
| N100300 | NeuroStem™ E18 Rat Hippocampal Progenitors | 1 x $10^6$ cells |
| N200300 | NeuroPure™ E18 Rat Cortical Progenitors | 2 x $10^6$ cells |

| | |
|---|---|
| **Shipping and Storage:** | NeuroFECT Transfection Reagent is shipped at room temperature. For maximum stability, store all reagents at 4°C upon receipt. If stored properly, the dried NeuroFECT reagent is stable for 12 months and the hydrated NeuroFECT reagent is stable for 6 months. The Hydration Buffer and DNA Diluent are stable for 12 months. |

**INTRODUCTION**: NeuroFect™ is a novel biodegradable cationic polymer created specifically for optimal transfection of neuronal cells. During transfection, the polymer/DNA complexes (polyplexes) are endocytosed into the cells, where the polymer is biodegraded into small non-toxic molecules. The ability of NeuroFect to biodegrade in vivo dramatically reduces its cytotoxicity and therefore maximizes the delivery of macromolecules into cells. NeuroFect is compatible with serum containing media, is easy to use, and provides the highest possible transfection efficiencies for your primary neurons.

## MATERIALS AND METHODS

### A. Preliminary Notes

I. The NeuroFECT Reagent is provided at a stock concentration of 5 µg/µl. You may use the reagent at the stock concentration or dilute it to 1 µg/µl for easier pipetting and transfection optimization. Use only sterile water or serum-free medium (such as OptiMem® I Medium, Invitrogen Corporation, Cat. # 31985-070) for dilution. If diluting with serum-free medium, NeuroFECT should be used within 30 minutes.

II. The following protocol was derived from optimizing transfection of Primary E18 Rat Hippocampal Neurons (Cat. # N100200) and Primary E18 Rat Cortical Neurons (Cat. # N200200). The DNA amounts, NeuroFECT:DNA ratios, cell plating densities, and timing of transfection may vary for other types of primary neurons and neuronal cell lines. However, the values given below for these parameters should function as good starting points. Optimization guidelines are provided on Page 2.

### B. Preparation and Growth of Primary Neurons

1. Seed neurons on freshly coated poly-lysine coated plates in the densities indicated in Table 1 using the following media for primary neurons: Neurobasal/B27, 0.5 mM glutamine (Add 0.25 µM glutamate for hippocampal neurons).

2. Incubate the cells at 37 ºC in 5% $CO_2$ for 3 days.

3. After 3 days, remove half of the plating medium volume per well and replace with same amount of the primary neuronal culture medium indicated in step 1 above. (Do not use glutamate at this point for hippocampal neurons.)

4. Continue culturing the cells for an additional 3-4 days

**Table 1: Cell Densities and Media Volume per Well**

| Primary Neuron Type | 24-well Plate | 96-Well Plate |
|---|---|---|
| Cortical Neurons | 80,000 cells/well | 20,000 cells/well |
| Hippocampal Neurons | 65,000 cells/well | 15,000 cells/well |
| Media Volume | 500 µl/well | 100 µl/well |


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### C. Transfection

5. Prepare the NeuroFECT and DNA in separate tubes according to Table 2. Dilute the NeuroFECT in half the Serum-Free Medium (SFM) volume indicated in Table 2 and your DNA in the other half of SFM, e.g., for Primary Hippocampal Neurons in 24-well plates, dilute 4 µg of NeuroFECT in 50 µl of SFM and 1.0 µg in 50 µl of SFM.

**Table 2: NeuroFECT, DNA, and SFM Amounts for Complex Formation in Primary Rat Hippocampal and Cortical Neurons**

| Neuron Type | 24-well plates (amounts per well) | | | 96-well plates (amounts per well) | | |
|---|---|---|---|---|---|---|
| | Reagent Amounts | | | Reagent Amounts | | |
| | NeuroFECT | DNA | SFM* | NeuroFECT | DNA | SFM* |
| Primary Hippocampal Neurons | 4 µg | 1.0 µg | 100 µl | 1.5 µg | 0.25 µg | 50 µl |
| Primary Cortical Neurons | 5 µg | 1.0 µg | 100 µl | 1.75 µg | 0.25 µg | 50 µl |

*SFM = Serum-Free Medium (Dilute the NeuroFect amount inidcated in half this volume and the DNA amount in the other half of this volume)

6. For NeuroFECT/DNA Complex Formation, add the reagents in this order: diluted NeuroFECT to diluted DNA in a drop wise fashion, and mix with gentle pipetting.

7. Incubate the NeuroFECT/DNA complex for 15-20 minutes at room temperature.

8. Remove the old culture medium from the cells and replace with the volume of fresh culture medium indicated in Table 1.

9. Add the NeuroFECT/DNA complexes to the cells. Gently mix by swirling plate.

10. Incubate the cells at 37 ºC in 5% $CO_2$.

11. Perform gene expression assay 24-48 hours later.

### D. Transfection Optimization Guidelines

12. To obtain the maximum transfection efficiency for other cell types besides primary cortical and hippocampal neurons, and for other tissue culture dish sizes besides 24-well and 96-well plates, we recommend using the above-mentioned DNA quantities and NeuroFECT/DNA ratios as starting points, and then testing different conditions according to the following guidelines:

**Table 3: Transfection Optimization Guidelines**

| Tissue Culture Dish | NeuroFECT: DNA Ratio | DNA | SFM* | Media Volume‡ |
|---|---|---|---|---|
| 96-well | 4:1 – 8:1 (µg:µg) | 0.1 – 0.5 µg | 50 ul | 100 µl |
| 24-well | | 0.5 – 2 µg | 100 ul | 500 µl |
| 6-well | | 2 – 6 µg | 500 ul | 1 ml |
| 60 mm | | 6 – 8 µg | 1 ml | 2.5 ml |
| 100 mm | | 8 – 12 µg | 2 ml | 5 ml |

*SFM = Serum-Free Medium (Dilute the NeuroFect amount inidcated in half this volume and the DNA amount in the other half of this volume)
‡ Volume prior to adding NeuroFECT/DNA Complexes

13. Optimization of cell plating densities for transfection of primary cells should start using densities giving maximum cell health during routine culturing. Greater or lessor densities can then be tested. Optimization of plating densities for transfection of neuronal cell lines should start at those giving 50-70% confluency on the day of transfection.

14. For addressing any cytotoxicity that may occur, it is recommended to reduce the amount of NeuroFECT reagent used in 15% increments until the cytotoxicity is eliminated.

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