Product Number(s): PN30075, PN30150

pn-Fect™ Transfection Reagent for Primary Neurons

Instruction Manual

Neuromics
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Purchaser Notification

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The purchase price paid for the pn-Fect™ Transfection Reagent kit by end users grants them a nontransferable, non-exclusive license to use the kit and/or its separate and included components (as listed in the Kit Contents section).

This kit is intended **for internal research only** by the purchaser. Such use is limited to the transfection of nucleic acid into neuronal cells as described in the product manual. Furthermore, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of Neuromics.

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This product is manufactured for Neuromics by Genlantis, a division of Gene Therapy Systems

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Kit Contents

Catalogue Number	Number of Tubes	Description	Size or Aliquot
PN30075	1 vial	pn-Fect™ Transfection Reagent, 75-300 rxns	750 µl
PN30150	5 vials	pn-Fect™ Transfection Reagent, 375-1500 rxns	750 µl

Shipping and Storage

The pn-Fect™ Transfection Reagent is shipped at room temperature. For maximum stability, store all reagents at 4°C upon receipt. If stored properly, all components are stable for 12 months.

Product Support

Telephone: 612-860-0130 OR 866-350-1500 (US toll free)
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Introduction

pn-Fect™ 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. pn-Fect is compatible with serum containing media, is easy to use, and provides the highest possible transfection efficiencies for your primary neurons.

pn-Fect Transfection Reagent Delivers:

• A New Unique Formulation
• Highest transfection efficiency for primary neurons
• Low cytotoxicity
• Simple, fast, and straightforward protocol

When compared to other commercially available transfection reagents, pn-Fect consistently offers superior transfection efficiencies for delivering macromolecules into Primary Neurons.

METHODS AND PROCEDURES

1. General Comments

1.1. For diluting DNA and pn-Fect, use serum-free medium only, such as OPTI-MEM® I Medium (Invitrogen Corporation, Catalog Number 31985-070).

1.2. To make a diluted stock of 1 µg/µl pn-Fect (as required for transfections in 96 well plates on Page 2), use sterile water only. NeuroFect that is diluted in serum-free medium is only good for

immediate use in the current experiment.
1.3. Alternatively, if you wish to calculate pn-Fect amounts needed per reaction, you can use the following formula:

DNA (µg) x Desired Ratio ÷ pn-Fect Concentration (µg/ul) = pn-Fect Amount (µl).
Example: 1.0 µg DNA x 6 (ratio or pn-Fect:DNA) ÷ 5 µg/ul pn-Fect = 1.2 µl pn-Fect.

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2. Cell Preparation and Growth

The protocol for the preparation of E18 rat hippocampal cells (Catalog Number PC35101) is provided as an example. For other neuronal cells or cell types, please refer to instructions provided by the vendor for optimal preparation and growth of cells.

2.1. Seed primary E18 rat hippocampal cells in plates coated with poly-D-lysine according to Table 1 using the following **plating medium**: Neurobasal/B27/0.5 mM glutamine/25mM glutamate Media (provided with all Neuromics' Primary Neurons).

Tissue Culture Plate Size	Cell Number	Medium Volume
24-well plates	65,000 cells/well	500 µl/well
96-well plates	15,000 cells/well	100 µl/well

Table 1: Cell Densities & Media Volumes Per Plate Size

2.2. Incubate the cells at 37°C in 5% CO_2 for 3 days.

2.3. After 3 days, remove half of the **plating medium** volume per well and replace with same amount of the following **culture medium**: Neurobasal/B27/0.5 mM glutamine.

2.4. Replace half the culture medium with fresh culture medium every 3-4 days as needed.

3. Transfection

For transfections in 24-well plates: The pn-Fect™ Transfection Reagent can be used directly without dilution at 5 µg/µl. To determine the amount of pn-Fect needed, use table 2 below. For best results, it is highly recommended to test all the pn-Fect:DNA ratios shown in Table 2, at least for the first transfection experiment.

pn-Fect:DNA Ratios (µg:ug)	µg DNA per well	µl pn-Fect (5 µg/ul) per well
4/1	1.0 µg DNA in 50 µl SFM*	0.8 µl in 50 µl SFM*
5/1		1.0 µl in 50 µl SFM*
6/1		1.2 µl in 50 µl SFM*
7/1		1.4 µl in 50 µl SFM*
8/1		1.6 µl in 50 µl SFM*

Table 2: pn-Fect and DNA Amounts and Volumes for 24-Well Plates
*SFM=Serum Free Medium

3.1. Prepare diluted pn-Fect and DNA in separate tubes as shown in Table 2.

3.2. Add **in this order**: diluted pn-Fect **to** diluted DNA in a drop wise fashion and mix with gentle pipetting.

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3.3. Incubate the pn-Fect/DNA complex for 15-20 minutes at room temperature to form polyplexes.

3.4. Remove old culture medium from cells and replace with 400 µl of fresh **culture medium.**

3.5. Add the 100 µl of pn-Fect/DNA complex to the cells in Step 3.4 above, for a total volume of 500 µl per well. Gently mix by swirling plate.

3.6. Incubate the cells at 37°C in 5% CO_2.

3.7. Perform gene expression or other desired assay 24-48 hours post transfection.

For transfections in 96-well plates*:* The pn-Fect Transfection Reagent needs to be diluted five fold **in sterile water** to a working concentration of 1µg /µl. If you plan on diluting the exact amount of pn-Fect needed for the current experiment, you can use serum free medium instead of water. To determine the amount of NeuroFect needed, use table 3 below. For best results, it is highly recommended to test all the pn-Fect:DNA ratios shown in Table 3, at least for the first transfection experiment.

pn-Fect:DNA Ratios (µg:ug)	**µg DNA per well**	**µl pn-Fect (5 µg/ul) per well**
4/1	.25 µg DNA in 25 µl SFM*	1.0 µl in 50 µl SFM*
5/1		1.25 µl in 50 µl SFM*
6/1		1.5 µl in 50 µl SFM*
7/1		1.75 µl in 50 µl SFM*
8/1		2.0 µl in 50 µl SFM*

Table 3: pn-Fect and DNA Amounts and Volumes for 96-Well Plates
*SFM=Serum Free Medium

3.8. Prepare diluted NeuroFect and DNA in separate tubes as in Table 3.

3.9. Add **in this order**: diluted pn-Fect **to** diluted DNA in a drop wise fashion and mix with gentle pipetting.

3.10. Incubate the pn-Fect/DNA complex for 15-20 minutes at room temperature to form polyplexes.

3.11. Remove old culture medium from cells and replace with 400 µl of fresh **culture medium.**

3.12. Add the 50 µl of pn-Fect/DNA complex to the cells in Step 3.11 above, for a total volume of 200 µl per well. Gently mix by swirling plate.

3.13. Incubate the cells at 37°C in 5% CO_2.

3.14. Perform gene expression or other desired assay 24-48 hours post transfection.

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