Product Number(s): PT30000, PT30002

pro-Fect™ Quick Single-Use Tubes

Instruction Manual

Neuromics
5325 West 74th Street, Suite 8
Edina, MN 55438
Phone: 952-374-6161
Fax: 612-677-3976
Email: pshuster@neuromics1.com
Website: www.neuromics.com

Purchaser Notification

Limited License

The purchase price paid for the pro-Fect™ protein delivery kit by end users grants them a non-transferable, 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.

Purchasers may terminate this License at any time by returning all pro-Fect protein delivery kit material and documentation to Neuromics, or by destroying all p-Fect Transfection Reagent kit components.

This document covers in full the terms of the p-Fect Reagent research only license, and does not grant any other express or implied license. The laws of Minnesota shall govern the interpretation and enforcement of the terms of this License.

Product Use Limitations

The pro-Fect protein delivery and all of its components are developed, designed, intended, and sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the handling of the kit components by following appropriate research lab practices.

For more information or any comments on the terms and conditions of this License, please contact:

Neuromics
5325 West 74th Street, Suite 8
Edina, MN 55438
Email: **phuster@neuromics.com**

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

Kit Contents

The pro-Fect™ protein delivery reagent is a unique lipid-based formulation that allows the delivery of proteins, peptides or other bioactive molecules into a broad range of cell types. The pro-Fect QuikEase kits contain 24 or 96 individual tubes of the BioPORTER reagent in a convenient, ready-to-use, single reaction format. Each tube contains sufficient material of the lyophilized pro-Fect reagent to perform 1 reaction in a 6-well plate (or 35mm dish) or 4 reactions in a 24-well plate (or 16mm wells).

The pro-Fect reagent is ready to use and only needs to be formulated with the protein solution (or other molecule of interest) to be delivered according to section 1.1 on page 7.

Catalog #s	# of Tubes	Description	Size or Amount
PT30000	24	pro-Fect Reagent, dried.	1 Reaction per Tube
	1	β-galactosidase control protein.	10ug at 100 µg/ml
	1	FITC-antibody control protein (fluorescein-labeled goat IgG)	10ug at 100 µg/ml
PT30002	96	pro-Fect Reagent, dried.	1 Reaction per Tube
	1	β-galactosidase control protein.	10ug at 100 µg/ml
	1	FITC-antibody control protein (fluorescein-labeled goat IgG)	10ug at 100 µg/ml

Stability and Storage

The pro-Fect reagent is shipped frozen. Upon receipt and for long-term use, store all reagent tubes at –20ºC. The reagent is stable at least for 1 year at the recommended storage temperature.

Introduction

The Pro-Fect reagent, is a versatile and efficient reagent for intracellular delivery of bioactive molecules, such as proteins, peptides or antibodies, into a broad range of cell types.

Although there are many effective reagents available to introduce transcriptionally active DNA into viable cells, approaches to deliver functional peptides and proteins into living cells are limited. For this reason, we have developed a unique protein delivery approach using a lipid-based carrier system. The resulting pro-Fect reagent is a novel composition that contains a proprietary reactive lipid mixed with other components.

This new approach is easy to use and more economical than both microinjection and electroporation for delivering biologically active proteins into living cells. The specific formulation of the pro-Fect reagent can deliver various molecules over a broad range of cell types in serum-free conditions.

Neuromics Antibodies • 5325 West 78th Street, Suite 8 • Edina, MN 55438
phone 952-374-6161 • fax 612-677-3976 • e-mail pshuster@neuromics1.com

The delivery is fast and the optimum delivery is usually reached after 3 to 4 hours of incubation. Various molecules such as fluorescent-antibody, high and low molecular weight dextran sulfate, phycoerythrin-BSA, β-galactosidase, caspase 3, caspase 8 and granzyme B have been successfully delivered into the cytoplasm of a variety of different adherent and suspension cells with pro-Fect reagent. Furthermore, apoptotic proteins such as granzyme B, caspase 3 or caspase 8 delivered into cells with the pro-Fect reagent are functional, since they can drive cells into apoptosis.

Now you can make your macromolecules directly available for a variety of studies like intercellular signaling, cell cycle regulation, control of apoptosis, study of oncogenesis, and transcription regulation to name a few. Our partners. Have extensively tested pro-Fect reagent and verified its effectiveness in delivering active molecules into a wide variety of cells. It is a powerful tool in the functional genomics and proteomics arsenal. In short, it is:

- Economical and easy to use
- Effective in multiple cell types (Table 1)
- Non-cytotoxic
- Fast and efficient. Optimum delivery obtained 3 to 4 hours after incubation
- A non-covalent comple
- Stable with an extended shelf life at -20^{o} C.

Table 1-Cell Types Testing Positive for pro-Fect			
HeLa-S3	NIH 3T3	K562	HeLa
BHK-21	CV-1	COS 7	P19
293	B16-F0	Jurkat	Hep G2
CHO-K1	COS-1	Ki-Ras 267 β1	MDCK

The pro-Fect Protein Delivery "Machine"

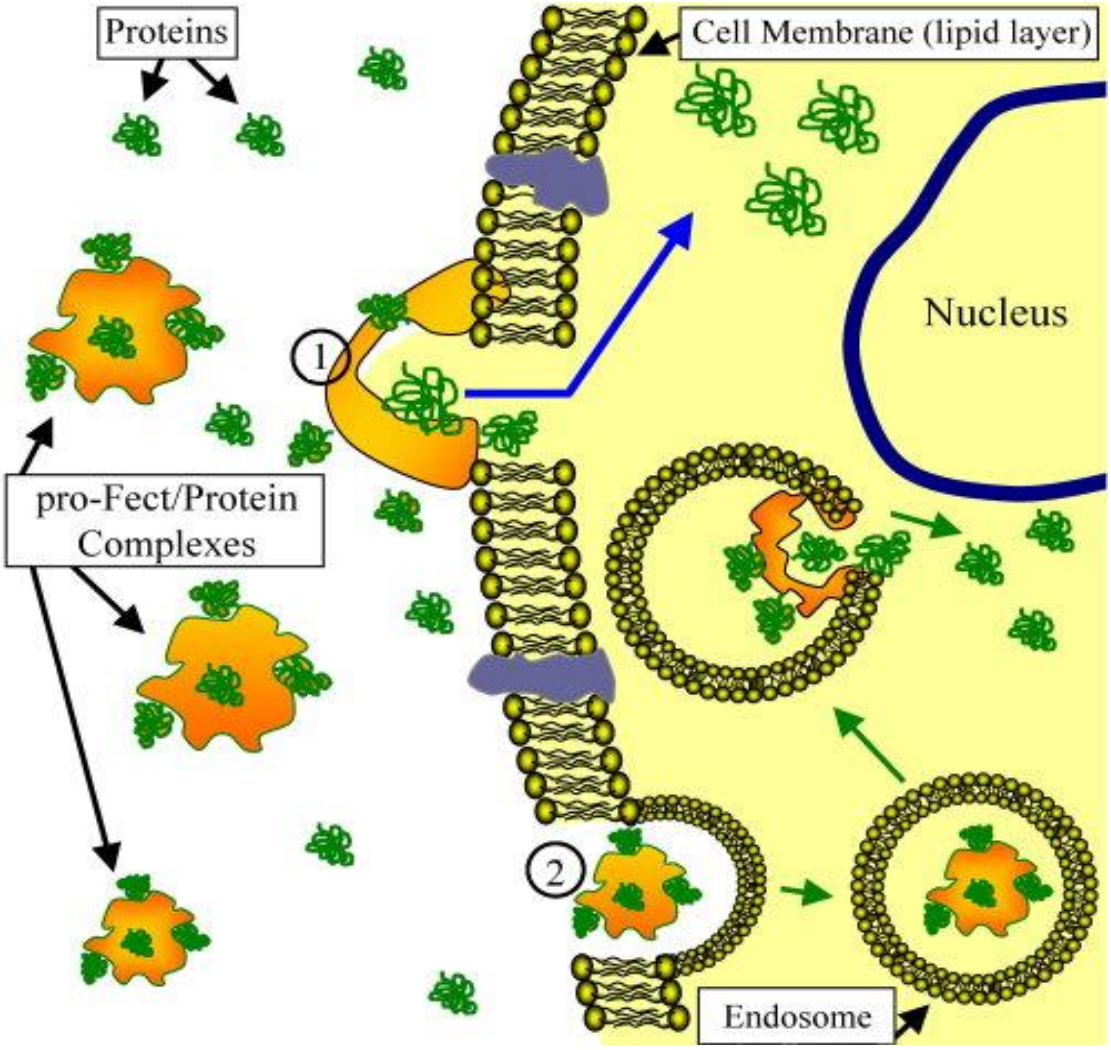
The dried pro-Fect reagent formulation is first dissolved in a solvent and aliquoted into small eppendorf tubes according to the type of assays conducted (see Methods and Procedures). After complete drying, pro-Fect is formulated with a solution of the protein or peptide to be delivered.

The pro-Fect reagent reacts quickly and interacts noncovalently with the protein, peptide or other molecules creating a protective vehicle for immediate delivery into cells. The hydrated mixture is then added onto cells, and the pro-Fect/protein complexes attach to negatively charged cell surfaces. It can then fuse directly with the plasma membrane and deliver the captured protein into the cells (see ① in Figure 1), or the pro-Fect/protein complexes are endocytosed by the cells and then fuse with the endosome releasing the pro-Fect-captured protein into the cytoplasm (see ② in Figure 1).

Delivery of molecules with the reagent is very easy and requires only 4 hours of incubation with the target cells.

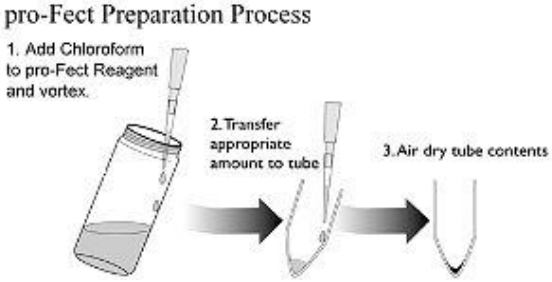
Neuromics Antibodies • 5325 West 78th Street, Suite 8 • Edina, MN 55438
phone 952-374-6161 • fax 612-677-3976 • e-mail pshuster@neuromics1.com

Figure 1: Delivery of Protein into Cell by pro-Fect



1. METHODS AND PROCEDURES

1.1. Preparation of the pro-Fect Reagent



1.1.2. Dissolve each tube containing the dry film of pro-Fect reagent with 250 µl of methanol or chloroform. Vortex for 10-20 seconds at top speed before each use.

CAUTION *Handle chloroform with caution by wearing the appropriate protective clothing and using a well ventilated space or hood. To avoid excessive evaporation of chloroform keep containers closed as much as possible.*

Neuromics Antibodies • 5325 West 78th Street, Suite 8 • Edina, MN 55438
phone 952-374-6161 • fax 612-677-3976 • e-mail pshuster@neuromics1.com

1.1.3. Pipette the desired amount of pro-Fect reagent into an eppendorf tube (see table below for suggested amount). Be sure to dispense the pro-Fect solution to the bottom of the tube. The amount of pro-Fect reagent needed varies depending on the type of experiment you are conducting (cell type, assay sensitivity, plate size, etc.). We recommend that you start by using 2.5 µl of pro-Fect reagent per reaction in a 24-well plate or 10 µl for a 6-well plate and vary the amount of protein to be delivered. We highly recommended that you optimize the delivery conditions by varying the amount of protein/peptide to be delivered first and then varying the amount of pro-Fect reagent, if necessary. Further optimization guidelines are offered in the Appendix section on page 10.

Table 2: Suggested Volume of pro-Fect reagent for Various Assays

Tissue Culture Dish	pro-Fect Volume	# of Reaction/Kit
96-well	1	240
24-well	2.5	96
12-well	5	48
6-well	10	24
60mm dish	20	12
100 mm dish	35	7

1.1.4. Leave the eppendorf tubes containing the pro-Fect reagent under a laminar flow hood to evaporate the solvent for at least **2 hours** at room temperature. For larger volumes, evaporate for at least 3-4 hours. Alternatively, you can use an inert gas like argon or nitrogen to gently and quickly evaporate the solvent by blow-drying. To avoid splashing the pro-Fect reagent, apply the gas flow gently and gradually. Make sure that the reagent stays at the bottom of the tube.
1.1.5. **Optionally**, you may vacuum the pro-Fect dry film for 1-2 additional hours to completely remove any trace of solvent.

IMPORTANT *The quality of the delivery reaction is severely affected by the presence of methanol or chloroform in the solution. Make sure that you follow the 2-4 hours recommendation for drying to assure the removal of all traces of solvent before adding your protein or peptide.*

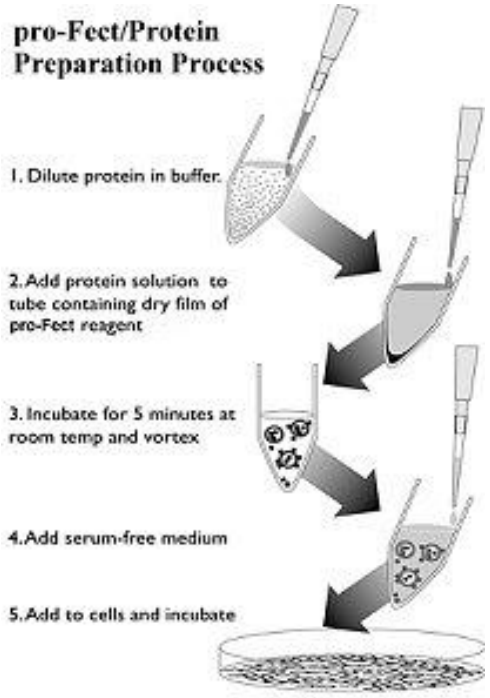
NOTE *We recommend that you aliquot the pro-Fect reagent immediately after dissolving in 250 µl of chloroform. This will help prevent extensive evaporation of the solvent and causing variations in reagent concentration. Dried Pro-Fect reagent can be safely stored at –20o C for at least one year without any substantial loss of activity.*

1.1.6. At this stage, you can proceed to the next step, preparation of the pro-Fect/protein complexes or store the tubes at –20°C until use.

1.2. Preparation of the pro-Fect /Protein Complexes

1.2.1. Dilute the protein, peptide, or other molecules in one of the following buffers: HBS (10 mM HEPES, 150 mM NaCl, pH 7.0) PBS (20 mM Na phosphate, 150 mM NaCl, pH 7.4) Tris Buffer (10 mM Tris, 150 mM NaCl, pH 7.0)

Neuromics Antibodies • 5325 West 78th Street, Suite 8 • Edina, MN 55438
phone 952-374-6161 • fax 612-677-3976 • e-mail pshuster@neuromics1.com



1.2.2. The final concentration of your proteins, peptides, or molecules of interest will vary according to their intrinsic properties and the type of assay performed. Further optimization guidelines are offered in the Appendix section on page 10.

For the following molecules listed in Table 3 below, we have found that the following concentration ranges yielded good delivery results.

IMPORTANT *Our experimental results suggest that some (though not all) highly positively charged molecules interact poorly with the pro-Fect reagent and are therefore not delivered into cells efficiently.*

Table 3: Protein concentration ranges used successfully for delivery	
Antibody, β-galactosidase or dextran sulfate	50-250 µg/ml
Caspase3	0.05 to 0.3 units/µl (165 to 1000 pg/µl)
Granzyme B	7.5 to 60 ng/µl

1.2.3. Use the diluted protein solution to hydrate the dried pro-Fect reagent. The amount of protein, peptide, antibody or other molecules to be delivered will depend on the type of experiment (cell type, assay sensitivity, plate size, etc.).

See Table 4 below for suggested amounts. Pipette up and down 3 to 5 times. Let stand at room temperature for 5 minutes then vortex gently and briefly (3-5 seconds) at low to medium speed.

Table 4 - Suggested Quantity of Proteins and Hydration Volumes

Tissue Culture Dish	Protein/ Ab, β-gal (µg)	Protein/ Caspase3 (ng)	Protein/ Granzyme B (µg)	Hydration Volume (µl) for pro-Fect
96-well	0.1-0.25	0.25-0.5	0.01-0.05	10
24-well	1-2	2-4	0.075-0.5	10-25
12-well	2-4	4-8	0.15-1	25-50
6-well	5-10	10-20	0.3-2	50-100
60mm	10-20	20-40	0.5-3	100-400
100mm	25-50	50-100	0.75-4	250-500

1.2.4. Add serum-free medium to the pro-Fect/protein complex to bring the final delivery volume up to the amounts recommended in Table 5 below.

Neuromics Antibodies • 5325 West 78th Street, Suite 8 • Edina, MN 55438
phone 952-374-6161 • fax 612-677-3976 • e-mail pshuster@neuromics1.com

Table 5: Suggested Cell Number and Transfection Volume

Tissue Culture Dish	Number of cells	Total Delivery Mix Volume
96-well	1-2 x 10^4	100 µl
24-well	0.5-1 x 10^5	250 µl
12-well	1-2 x 10^5	500 µl
6-well	2-4 x 10^5	1 ml
60 mm	5-10 x 10^5	2.5 ml
100 mm	10-20 x 10^5	5 ml

1.2.5. Aspirate medium from the cells to be tested, wash once with serum-free medium (optional) and then transfer the final delivery mix onto cells.
1.2.6. For **adherent cells**, directly add the pro-Fect/protein complexes (resuspended in serum-free medium) onto the washed cells. For **suspension cells**, first count the cells, centrifuge them at 1200 rpm for 5 minutes, and then resuspend them in serum-free medium. Adjust their concentration according to the size of your plate or dish and your transfection volume. Pipette the cell suspension into the tube of pro-Fect/protein mixtures and then transfer it to your well or dish.
1.2.7. Incubate for 3-4 hours at 37° C. If longer incubation time is required, add one volume of 20% serum-containing medium directly to the well or dish. It is not necessary to change the medium after the initial serum-free incubation. However, if incubation times that are longer than 24 hours are necessary, medium can be replaced as needed.

NOTE *The presence of serum in the first hours of incubation is inhibitory for delivery. Make sure that the first 3-4 hours of incubation are done in serum-free conditions followed by growth in serum-containing medium.*

1.2.8. Proceed with your experiment for observation or detection assays. Cells can be fixed or can be observed alive.
1.2.9. Two positive controls are provided in the Kit. The fluorescein-antibody and β-galactosidase can be tested as described below in the example protocols.

2. EXAMPLE PROTOCOLS

2.1. Delivery of a Fluorescent Antibody, β-galactosidase, or dextran sulfate (High and low Molecular Weights) for 24-well plates (or 22 mm cover slips).

2.1.1. Seed 0.5 to 1 x 10^5 cells per well in a 24-well plate or on a cover slip and let grow overnight
2.1.2. Pipette 2.5 µl of pro-Fect reagent (dissolved in 250 µl of methanol or chloroform) into the bottom of each eppendorf tube. Evaporate the solvent under a laminar flow hood for 2 hours (or blow dry with an inert gas). Optionally, vacuum the tube for 2 hours to completely remove any trace amount of solvent. Proceed to the next step (2.1.4) or store the tubes at –20°C until use.
2.1.3. Dilute 0.5-2 µg of FITC-Ab, dextran sulfate, or β-galactosidase in 10 to 25 µl of HBS or PBS. For β-galactosidase we recommend using PBS (buffer formulas available in section 1.2.1). The FITC-Ab and β-galactosidase control proteins provided in the kit are ready to use without further manipulation. Just thaw and mix them well before use.

Neuromics Antibodies • 5325 West 78th Street, Suite 8 • Edina, MN 55438
phone 952-374-6161 • fax 612-677-3976 • e-mail pshuster@neuromics1.com

2.1.4. Hydrate the pro-Fect reagent dry film with 10-25 µl of the diluted protein solution. Pipette up and down 3 to 5 times. Incubate at room temperature for 3-5 minutes, then vortex briefly and gently at low to medium speed for few seconds.
2.1.5. Bring the final volume of the pro-Fect/protein mixture to 250 µl with serum-free medium.
2.1.6. For 24-well plates, aspirate the medium from the cells to be tested, wash once with serum-free medium (optional) and then transfer the pro-Fect/protein mixture directly onto the cells.

Note *If cover slips are used, blot the cover slip dry and place it in a 35-mm dish. Transfer the pro-Fect/protein mixture directly onto the cells.*

2.1.7. Incubate cells in a 5% CO_2 incubator at 37°C for 4 hours. Add 250 µl (1 volume) of 20% serum-containing medium directly to the 24 well if the incubation time needs to be longer than 4 hours.
2.1.8. For cover slips, add 1 to 2 ml of growth medium to the 35-mm dish containing the cover slip if the incubation time needs to be longer than 4 hours.
2.1.9. After the incubation, wash the cells twice with PBS and proceed with the appropriate assay.

Fluorescent microscopy: after washing, cells growing on cover slips are mounted directly onto a hanging drop slide with PBS. Living cells are then directly observed under a microscope. Alternatively cells can be fixed for observation.

β-galactosidase assay (X-Gal staining): for all of our assays we have used the Gene Therapy Systems X-Gal staining Kit (cat # A10300K, Genlantis), with the brief protocol on the left.

B-galactosidase Assay Sample Protocol

1. Aspirate medium 4 to 24 hrs after β-gal delivery.
2. Wash cells twice with PBS (500 µl).
3. Fix cells with the 1x fixing solution (250 µl) for 10 min at room temperature.
4. Prepare the staining solution.
5. Remove the fixing solution and gently wash cells 2 x with PBS (500 µl)
6. Add the staining solution (250 µl) and incubate 2 hrs to overnight at 37° C
7. Remove the staining solution. Wash cells with PBS and examine under a light microscope. Calculate percentage of stained cells if desired.

2.2. Delivery of a Fluorescent Antibody, β-galactosidase, or dextran sulfate (High and low Molecular Weights) for a 6-well plate or 35 mm dish.

2.2.1. Seed 2 x 10^5 cells in 6-well plate and let grow overnight.
2.2.2. Pipette 10 µl of pro-Fect reagent (dissolved in chloroform or methanol) into the bottom of each eppendorf tube. Evaporate the solvent as described in section 2.1.2 above.
2.2.3. Dilute 5-10 µg of protein in 50-100 µl of appropriate buffer as in section 2.1.3 above.
2.2.4. Hydrate the pro-Fect reagent dry film with 50-100 µl of the diluted protein solution. Pipette up and down 3 to 5 times. Incubate at room temperature for 5 minutes, then vortex briefly and gently at low to medium speed.
2.2.5. Bring the final volume of the pro-Fect/protein mixture to 1000 µl with serum free medium
2.2.6. Aspirate the medium from the cells to be tested, wash one time with serum-free medium (optional) and then transfer the pro-Fect/protein mixture directly onto the cells.
2.2.7. Incubate cells in a 5% CO_2 incubator at 37°C for 4 hours. If incubation time needs to be longer than 4 hours, add 1000 µl (1 volume) of 20% serum containing medium directly to the well.

2.3. Delivery of granzyme B and caspase 3 into Jurkat or Ki-Ras-267 β1 cells for 24-well plates.

2.3.1. For adherent cells such as Ki-Ras-267 β1 (prostate cancer cell line) seed 0.5 x 10^5 in 24-well and let grow overnight. For Jurkat cells see section 2.3.6.
2.3.2. Pipette 2.5 µl of pro-Fect reagent (dissolved in chloroform or methanol) into the bottom of each eppendorf tube. Evaporate the solvent as described above (section 2.1.2)

Neuromics Antibodies • 5325 West 78th Street, Suite 8 • Edina, MN 55438
phone 952-374-6161 • fax 612-677-3976 • e-mail pshuster@neuromics1.com

2.3.3. Dilute caspase 3 at 330-660 pg/µl or granzyme B at 15-45 ng/µl in HBS buffer. Buffer formula is available in section 1.2.2.
2.3.4. Hydrate the pro-Fect reagent dry film with 10 µl of the diluted protein solution. Pipette up and down 3-5 times. Incubate at room temperature for 3-5 minutes; vortex briefly and gently at low/medium speed for few seconds.
2.3.5. For **adherent cells** such as Ki-Ras-267 β1, bring the final volume of the Pro-Fect/protein mixture to 200 µl with serum-free medium. Aspirate the medium from the cells to be tested, wash once with serum-free medium (optional) and then transfer the pro-Fect/protein mixture directly onto the cells.
2.3.6. For **suspension cells** such as Jurkat, count and pellet the cells, resuspend them in serum-free medium to 0.5 x 106 cells/ml. Add 200 µl of the cell suspension to the pro-Fect/Protein mixture and then transfer the mix to a 24-well plate.
2.3.7. Incubate cells in a 5% CO_2 incubator at 37°C for 4 hours. Add 1ml of serum-containing medium directly to the well and incubate overnight.
2.3.8. The next day, proceed with the apoptosis assay using any commercially available annexin Vpropidium iodine labeling kit. This assay can also be at time points earlier than 4 hours (see brief sample protocol on the left).

Apoptosis Assay Sample Protocol

1. Transfer medium and cells (after mild trypsinization for adherent cells) to 13 x 75mm plastic tubes. Wash wells with some serum-containing medium, pool them together and centrifuge at 1400 rpm for 5 minutes.
2. Wash cells with 500 µl of cold PBS without disturbing the pellet, centrifuge at 1000 rpm for 3 minutes.
3. Resuspend cells in 100 µl of cold annexin V binding buffer.
4. Add annexin V-FITC and propidium iodine (PI) to your samples and incubate at room temperature according to the instructions of the annexin V-PI labeling kit manufacturer.
5. Analyze your samples as soon as possible by flow cytometry or fluorescence microscopy.

APPENDIX

Protocol for Optimization

It is highly recommended to optimize your conditions in order to get the best performance for the Pro-Fect reagent. Several parameters can be optimized as follows:

- Amount of protein, peptide or other molecules to be delivered.
- Hydration buffer containing the diluted protein solution.
- Amount of Pro-Fect reagent.
- Concentration of the protein solution during the preparation of the complexes.
- Hydration volume for Pro-Fect reagent.
- Cell types and cell culture density.
- Time of incubation.

Many of these factors have been investigated at Gene Therapy Systems during the research and development of the pro-Fect reagent. We recommend that you optimize one parameter at a time using the suggested conditions in the Methods and Procedures section.

1. Start by using a fixed amount of the pro-Fect reagent, for example use 2.5 µl of Pro-Fect reagent per reaction in a 24-well plate.
2. Vary the amount of protein to be delivered. Use a standard buffer to do so, for example HBS or PBS. Depending on the sensitivity of the endpoint assay, a greater amount of protein and pro-Fect reagent may be required.

Neuromics Antibodies • 5325 West 78th Street, Suite 8 • Edina, MN 55438
phone 952-374-6161 • fax 612-677-3976 • e-mail pshuster@neuromics1.com

3. If further optimization is required, fix the concentration and amount of protein/peptide to be delivered and vary the quantity of prfo-Fect reagent (see table below). The reagent interacts with your molecules of interest via hydrophobic and electrostatic interactions, and because each molecule will have different charge and hydrophobicity, the amount of pro-Fect reagent may need to be changed. Although Pro-Fect reagent is not cytotoxic at the recommended concentrations, it may show some signs of cytotoxicity at higher reagent to cells concentration ratios.

Tissue Culture Dish	Pro-Fect range (μl)
96-well	0.25-1.5
24-well	1.25-5
12-well	2.5-7.5
6-well	5-15
60mm dish	15-30
100mm dish	25-45

4. After you identify the correct amount of pro-Fect reagent and protein to be used for the delivery reaction, you can then optimize the volume used to hydrate the pro-Fect dry film (step 1.2.3). To test this parameter, fix the protein amount and vary the hydration volume for pro-Fect reagent (see Table 4 in section 1.2.3).
5. Different dilution buffers for your protein solution such as Tris, HBS, and PBS buffers can be tested. We have found that for some molecules the buffer composition may be critical. Indeed, with β-galactosidase the efficiency is very good with PBS buffer but not with Tris buffer. Whereas with dextran sulfate HBS is the right buffer. Also, apH may also be critical for some molecules because of their different charge and hydrophobicity; varying the pH may help interaction with the pro-Fect reagent.
6. At this point the cell number can also be optimized since the delivery efficiency may be sensitive to the confluency of the cells in culture.
7. Depending on the type of functional assay performed, shorter or longer incubation time may be necessary. If aggregation of the pro-Fect/protein complexes occurs during optimization (seen as large glowing particles under the microscope), try one or any combination of the following recommendations:

- Briefly sonicate the Pro-Fect/protein mixture.
- Increase the Pro-Fect hydration volume.
- Lower the concentration of protein or molecule.
- Lower the amount of Pro-Fect reagent.

Neuromics Antibodies • 5325 West 78th Street, Suite 8 • Edina, MN 55438
phone 952-374-6161 • fax 612-677-3976 • e-mail pshuster@neuromics1.com

Quality Control

To assure the performance of each lot of the pro-Fect reagent, we qualify each component using rigorous standards. The following assays are conducted to qualify the function and activity of each kit component in living cells.

Kit Component	Quality Control Standard
Pro-Fect Reagent	1. Efficient FITC-antibody delivery in NIH-3T3 cells. 2. Efficient β-galactosidase delivery efficiency in NIH-3T3 cells. 3. Induction of apoptosis in Jurkat cells using granzyme B and caspase3. Delivery efficiency is assayed by monitoring the percentage of cells that become apoptotic through flow cytometry. 4. Testing for absence of bacterial and fungal contaminants.
FITC-Antibody Positive Control	1. Analysis by gel electrophoresis and measurement of fluorescence. 2. Testing for intracellular delivery by the pro-Fect reagent in NIH-3T3 cells.
β-galactosidase Positive Control	1. Testing for intracellular delivery by the pro-Fect reagent in NIH-3T3 cells.

Troubleshooting Guide

Problem	Possible Causes	Recommended Solutions
Low delivery efficiency	Reagent Solubility	− Make sure you use chloroform or methanol to solubilize pro-Fect reagent. − Vortex vigorously.
	Aliquoting of the Reagent	Be sure to aliquot the pro-Fect reagent to the **bottom** of the eppendorf tube
	Drying of the Reagent	− Use sufficient time to air dry or vacuum dry pro-Fect reagent. − If used inert gas to dry the reagent, make sure that pro-Fect reagent did not splash on the side of the tube.
	Amount of Reagent	Vary the amount of reagent as recommended in the optimization protocol.
	Protein/peptide concentration	Titrate the concentration and the hydration volume of pro-Fect reagent.
	Hydration buffers.	Change the protein dilution buffer and/or the pH to improve delivery.
	Mixing of pro-Fect reagent and protein.	Allow the mixtures to form for at least 3 minutes. Mix well by pipetting up and down; **do not vortex *vigorously* at this step.**
	Charge of Molecules to be delivered.	Highly positively charged molecules are difficult to deliver with pro-Fect reagent. Modify the hydration buffer or pH to change the charge of the molecules.
	Unknown properties of molecules to be delivered.	Mix a fluorescent molecule or directly label the protein of interest in order to monitor delivery.
	Cell Density.	Use cells that are 50-60% confluent.
	Wrong medium used.	Make sure to use serum-free medium during the first hours of delivery.
	Improper storage.	Pro-Fect reagent is very stable but long exposure to elevated temperatures may cause degradation. Store pro-Fect reagent at –20° C.
	Time of incubation.	Incubate pro-Fect/protein complexes with cells for at least 3-4 hours.
	Type of cell line used.	Test BioPORTER reagent with the positive controls in parallel with cell lines that were successfully used (see Table 1 on page 4 for cell line suggestions).

Neuromics Antibodies • 5325 West 78th Street, Suite 8 • Edina, MN 55438
phone 952-374-6161 • fax 612-677-3976 • e-mail pshuster@neuromics1.com

Problem	Possible Causes	Recommended Solutions
Aggregation	Amount of pro-Fect reagent used.	Too much reagent could cause aggregation; lower the amount of Pro-Fect reagent if you think you used an excess amount.
	Evaporation of the Pro-Fect stock solution.	Excessive evaporation of the dissolved pro-Fect reagent will change its concentration. Titrate down or use lower amounts of reagent.
	Storage of the pro-Fect/ protein complexes.	Pro-Fect/protein complexes should be freshly prepared. If complexes have been prepared and stored for too long, aggregation may occur
Cytotoxicity	Excess pro-Fect reagent.	Decrease the amount of reagent used.
	Molecules delivered are toxic.	− Use the appropriate control reactions like cells alone, pro-Fect reagent alone, "control" or "safe" protein alone, and your molecule(s) of interest alone. − Check the purity of the molecule of interest to be delivered.
	Unhealthy cells.	− Check cells for contamination. − Thaw a new batch of cells. − Cells are too confluent or cell density is too low. − Check the culture medium (pH, kind used, last time changed, etc.) − Check materials used for proper function (culture plates, incubator temperatures, etc.)

Do not hesitate to contact me with questions.

Pete Shuster, CEO
pshuster@neuromics.com
Direct phone: 612-801-1007

Neuromics Antibodies • 5325 West 78th Street, Suite 8 • Edina, MN 55438
phone 952-374-6161 • fax 612-677-3976 • e-mail pshuster@neuromics1.com