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Phone: (866)-620 4018, (619)-562-1518
Fax: (619)-562-1326 • info@targetingsystems.net
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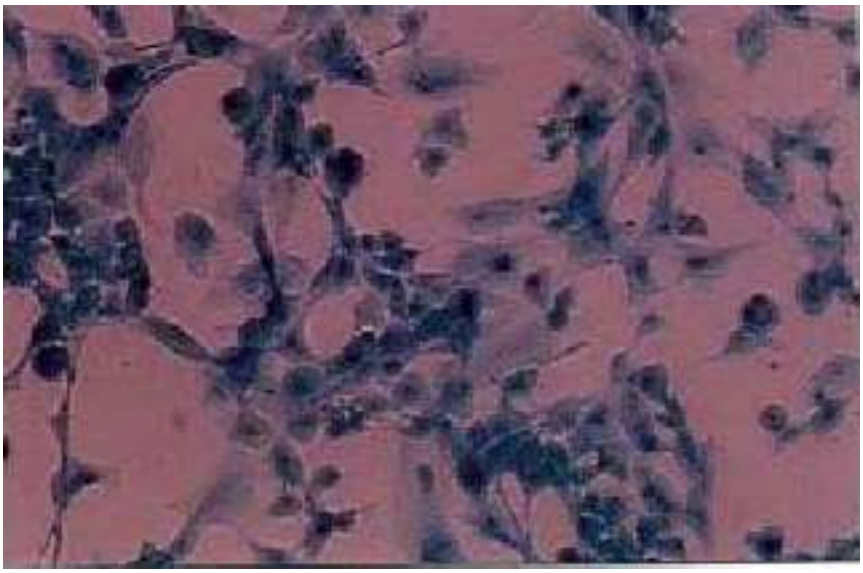
PROFECT PROTEIN DELIVERY REAGENTS

PRODUCT APPLICATIONS GUIDE 2010



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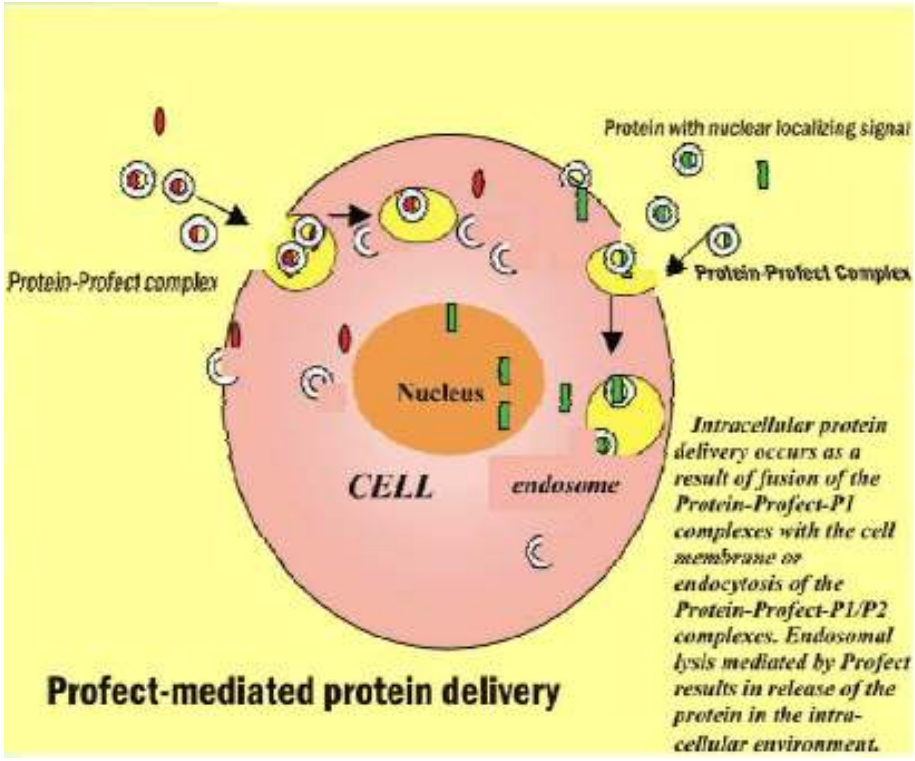
Profect Reagents for efficient protein delivery

Profect-P1
A lipid reagent that forms non-covalent complexes with proteins and enables translocation of intact functional proteins across the cell membrane.

Profect-P2
A non-lipid reagent that forms non-covalent complexes with proteins and enables protein transport across both the cell membrane as well as the nuclear membrance. Profect-P2 has endosmolytic properties which protect the intemalized proteiin from being degraded in the lysosomes, Profect-P2 also has the unque ability to escort both DNA and protein across the nuclear membrane.

Profect-P1 and Prfect-P2 can form non-covalent complexes with a variety of proteins and can be used to successfully co-deliver different proteins. Proteins delivered with Profect range from 10 Kd to 540 Kd.



Applications

- Delivery of enzymes, antibodies and peptides Study signal transduction Study transcriptional regulation of gene expression
- Study protein interactions in living cells.
- Screening peptide libraries
- Study host-parasite interactions and identification of virulence factors.
- Protein half life studies
- Studying identify proteins/antibodies affecting apoptsis, anglogenesis, tumorigenesis

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Why Profect?

Efficient delivery of intact proteins: The most important property of the Profect reagents is that they enable highly efficient delivery of intact, functional proteins into many difficult-to-transfect primary cell types and several cell lines.

Versatility: These reagents have been used to successfully deliver a variety of [proteins (11,000 Kd to 540,000 Kd) into a variety of primary cell lines.

Compatibility with Cell culture media and antibiotics: The reagents are compatible with transfection to physiological buffer s such as those involving signal transduction cannot be carried out in OptiMEM1 or media with growth factors as these influence signal transduction

Site-specific protein delivery: The Profect reagents provide a mechanism for site-specific protein delivery (e.g. nuclear delivery) in many instances. This is important in cases where it is desirable to target the protein to a desired sub cellular organelle. Nuclear delivery is effected by using the Porfect P2 reagent and made more efficient by co-delivering the protein (e.g. IgG) with histone to target the nucleus. Similarly targeting to other organelles can often be accomplished by co-delivering the protein of interest with a protein that localizes to the organelle of interest.

The ability of Profect reagents to mediate efficient protein transfection was first tested using β-galactosidase (540Kd) as a reporter protein. In these experiments 100 ngs of β-galactosidase was complexed with 5 ìl of the Profect reagent (Profect-P 1 or 2) in 500 ìl of PBS and used to transfect cells in 12-well dishes. The cells were exposed to the protein-Profect complexes for 1 hr at 37 °C, then washed twice with PBS, fixed and stained for visualization of β-galactosidase activity. All 4-cell types tested (NIH 3T3, Hela, retinal pigmented epithelial cells and human lens epithelial cells) showed efficient delivery of β-galactosidase (85-100%, data displayed on our website)

An important requisite for versatile application of a protein delivery reagent is the ability to control the amount of protein delivered into the cells. To test this, Hela cells were transfected with either 600 ngs or 3 ìg of β-galactosidase using the Profect P-2 reagent. As shown in Fig. 2, 100 % of cells were transfected with the β-galactosidase protein. Cells transfected with 3 ìg β-galactosidase (right panel) showed higher activity than cells transfected with 600 ngs β-galactosidase (left panel) indicating that it is possible to control amount of protein delivered into cells by manipulating the amount of protein used for transfection.

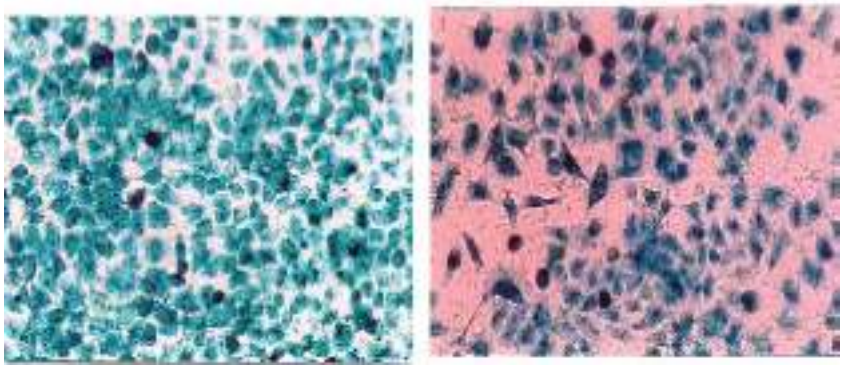

Figure 1a: Transfection of Hela cells with B - galactosidase: Cells were transfected with 0.6 µg (left)or 3 µg (right panel) of β -galactosidase using the Profect-2 reagent. Data courtesy of Drs Frank Stenner-Liewen and JuanZapata, Dr J Reed's lab, Burnham Institute, La Jolla

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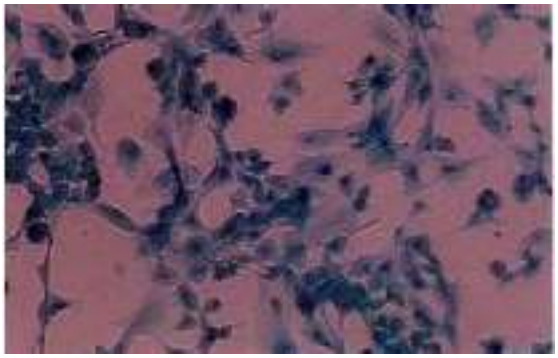

Figure 1b: Retinal pigmented epithelial cells; human lens epithelial cells and Hep 3B cells. Retinal pigmented epithelial cells and human lens epithelial cells used in these studies were kindly provided by Dr Naseem Ansari, Univ. of Texas Med. Branch, Galveston, TX.

Profect-mediated Antibody Transfer:

Efficiency Of Antibody Delivery Using Profect-p1 And Profectp2 -facs Analysis: The ovarian cancer cell line OVCAR-3 was used to assess the efficiency of Profect-mediated antibody delivery. Cells were attached to six-well plates at a cell number of 1x10e5 cells/well and transfected with FITC-labeled anti-β-actin monoclonal antibody (1.5 µg/ml; Sigma Chemical Co., St Louis, MO) using P1 and P2 reagents (2.5 µl/ml). Negative controls included untreated as well as cells incubated with FITC-labeled anti-β-actin antibody, with P1 reagent, or with P2 reagent, respectively. Cells were detached with EDTA-based dissociation solution (Cell and Molecular technologies, Inc., Phillipsburg, NJ), washed with PBS and fixed in 10% fixative in wash buffer (Intergen Company, Purchase, NY). Subsequent FACS analysis was performed using a Beckton Dickinson FACScan Plus instrument (Beckton-Dickinson, Mountain View, CA). The figure shows the transfection efficiency of a FITC-labeled anti-β-actin antibody in OVCAR-3 ovarian cancer cells and using the Profect reagents. Incubation with antibody alone did not cause a significant increase of fluorescence intensity compared to untreated cells (see figure, panel A). Statistical analysis showed that P1 reagent transduced 84% of cells with anti-b-actin antibody (panel B, middle) leading to a significant histogram shift (panel B, bottom). P2 reagent's transfection efficiency was assessed to be 94% of treated cells (panel c, middle). Both Profect reagents achieved high protein transfection rates in ovarian cancer cells, which are notoriously hard to transfect using standard gene delivery reagents.

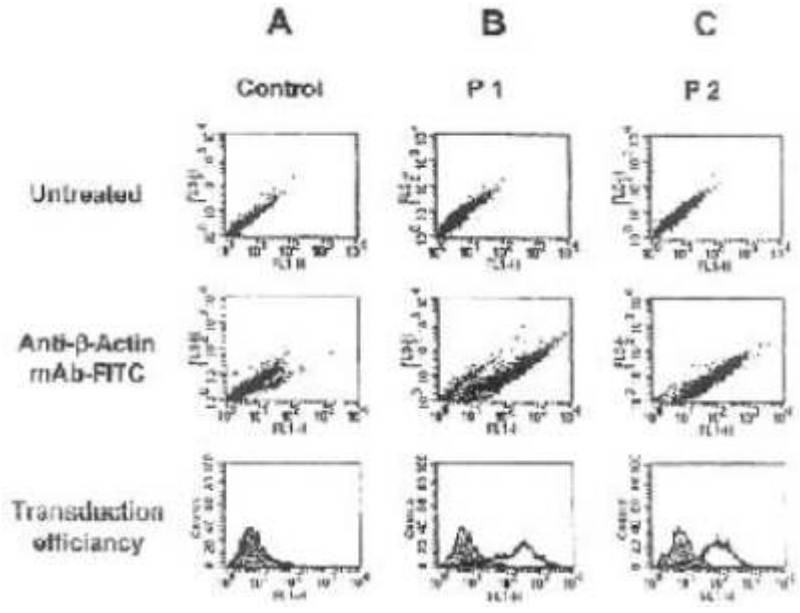

Figure 3: Transfection of β-Actin antibody with Profect reagents analyzed by FACS. (A) Data courtesy of Drs Frank Stenner-Liewen and Juan Zapata, Dr J Reed's lab, The Burnham Institute, La Jolla

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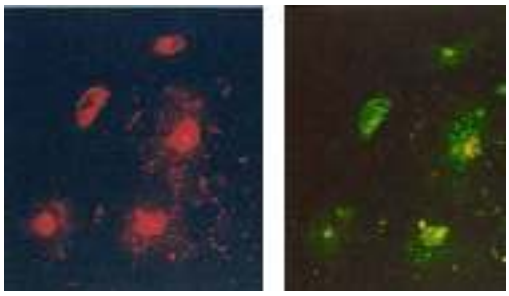
Data On Profect-mediated Antibody Delivery Into Different Cell Types:

(All Alexa-conjugated proteins were a kind gift from Molecular Porbes Inc, Eugene, OR). J Reed's lab, The Burnham Institute, La Jolla



An important application of antibody delivery is that the antibody may need to go to a specific site within the cell such as the nucleus. We have shown below (figure 4) that an alexa-594- conjugated antibody can be delivered to the nucleus of Cos-7 cells using the Profect-P2 reagent when it is co-delivered with alexa-488-conjugated histone H1

Figure 4: Co-delivery of an Alexa-594 conjugated antibody with Alexa 488-conjugated histone into Cos-7 cell using Profect-P2

Are proteins delivered using the Profect reagents functional?

An important requisite for an efficient protein delivery systems is that proteins delivered should retain their normal physiological functions. In an effort to demonstrate the efficacy of the Profect reagents to deliver we transfected CV-1 cells and MCF-7 cells with active caspase 3 and examined the cells for apoptosis using phase contrast microscopy combined with DAPI staining (in case of CV-1 cells) or assessed apoptosis with the help of the Vybrant apoptosis assay kit (in case of MCF-7 cells) kindly provided by Molecular Probes, OR. In these experiments the caspase-transfected CV-1 cells were also stained with the nuclear stain DAPI and examined by fluorescence microscopy to assess condensation and fragmentation of the nucleus that is a characteristic of caspase induced apoptosis. MCF-7 cells transfected with caspase were exposed for 30 minutes to a combination of two dyes (Yopro, and propidium iodide) in the vybrant apoptosis assay kit. Approximately 3-4 hrs post-transfection and then examined by fluorescence microscopy (Fig 8B). The results of this experiment (Fig.8A) suggest that transfection with active caspase 3 shows extensive cell death (top panel, phase contrast image) together with condensed, fragmented nuclei (top panel, DAPI staining) whereas cells transfected with a control protein (B-galactosidase) using the Profect -P2 reagent show intact nuclei (DAPI staining, lower panel) and healthy cells (phase contrast image, lower panel). The results of the apoptosis experiment in MCF-7 cells (fig 8B) show that MCF-7 cells transfected with active caspase using Profect p-2 showed extensive apoptosis (bright yellow cells) whereas cells transfected with caspase in the absence of Profect did not show any fluorescence.

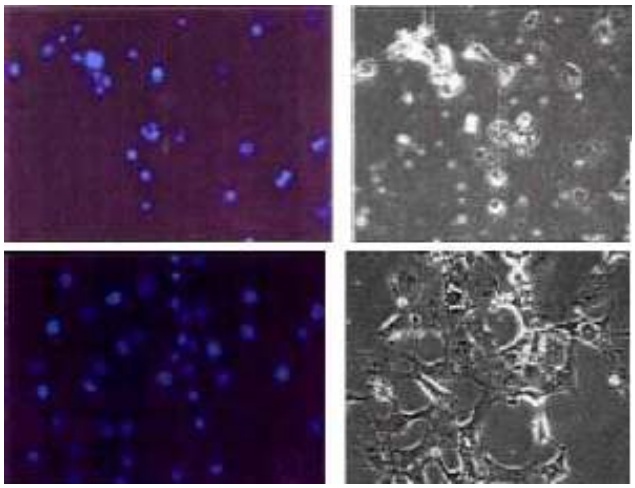


Figure 8A: Caspase-mediated apoptosis in CV-1 cells: CV-1 cells were transfected with 50 ngs of active caspase 3(top panel) or with a control protein, B-galactosidase(lower panels) using 5 ul Profect-P2. The left hand panels shwo DAPI staning of the nuclei and the right hand panels show phase-contrast images of the transfected cells.

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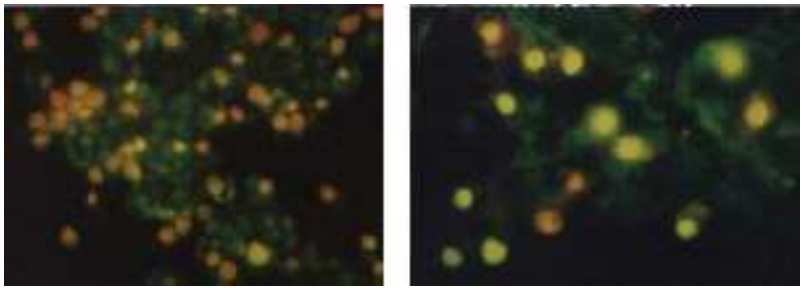

Figure 8B: Caspase-mediated apoptosis in MCF-7 cells: Apoptotic cell are seen as bright yellowish green fluorescent cells due to entry of Yopro into apoptotic cells. Orange cells indicate late apoptosis

Profect Protocol

The protocol is provided as an example for a 6-well plate format. Amounts for other plate sizes are given in Table 1.
Vortex the Profect P-1 reagent at full speed for 30 seconds once, just before use. Store the Profect-P-1 reagent at 20 °C. The Profect-P2 reagent can be stored at 4 °C or at -20 °C.

Protocol:

1. Set up cells to be transfected in Labtek-chamber slides so that they are about 80% confluent at the time of the experiment.
2. Add 0.5-5 µl of protein solution (100 ng to 10 µg, in general we recommend 5 µg) to a sterile tube containing the 100 ul of serum-free DMEM
3. Add 3 µl or 5 µl of Profect reagent (mix well before use)
4. Vortex for 15 seconds.
5. Incubate at room temperature for 20 minutes
6. Diilute transfection complexes with DMEM to 1 ml
7. Remove serum-containing growth media from cells by aspirating, wash cells with serum-free medium and add 1ml of serum-free medium to each well.
8. Add the transfection complex mixture to cells
9. Return plate to incubator and incubate for 2-5 hours.
10. Add 1 ml of complete media (containing 10% serum) to each well.
11. Replace media on the following day and continue incubation until assaying. Wash cells with serum-free medium before assaying to remove any untransfected Protein.

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*The transfection complex mixture is composed of protein and Profect Transfection Reagent in serum-free medium. For example, at the incubation step (step 8) (6-well format), transfection complex mixture consisting of 2 ìg protein, and 3ìl Profect Transfection Reagent in 200 ìl serum-free medium is added to a well containing cells in a 1 ml volume.

Table 1: Protein transfection in different plat formats

Culture Vessel	Volume of Plating Medium (per well)	Protein (µg) In serum-free medium	Profect transfection complex mixture*
96 well	100 µl	0.1-0.2 µg in 10 µl	0.2 µl
24 well	200 µl	0.2-1.0 µg in 50 µl	0.2-2 µl
12 well	0.5 ml	0.5-2.0 µg in 100 µl	0.4-4 µl
35 mm dish	1 ml	0.5-2.0 µg in 100 µl	1-10 µl
6 well	1 ml	0.5-2.0 µg in 100 µl	1-10 µl
60 mm dish	2 ml	2.0-12 µg in 0.5 ml	4-24 µl
10 cm dish	7 ml	6.0-30 µg in 1.0 ml	6-60 µl

Peptide Delivery: Suggested protocol for peptide delivery:

Mix 6.5 ìg peptide with 5 ìl of the P-2 reagent in 100 ìl of high glucose DMEM. Mix well and incubate at room temperature for 20 minutes then Vortex for 15 seconds. Dilute the complexes to 1 ml with high glucose DMEM and follow the transfection protocols recommended above.

For 96 well plates: Mix well and add 40 ìl of complex per well of a 96 well plate (aspirate culture media before addition of transfection complex.) Incubate at 37 °C for 3 hrs. Add 100 ìl of complete media and continue incubation. Wash cells 4 times with serum free media and assay.

Troubleshooting:

Toxicity may be observed when using low cell densities, or very small amounts of protein. Usually an excess of uncomplexed Profect reagent may show some toxicity so if toxicity is observed when transfecting with very small amounts of protein (less than 100 ng) we recommend adding a carrier protein (e.g. β-galactosidase) so that the final protein concentration is about 2 ìg total protein complexed with 5 ìl of Profect. Another alternative is to use lesser amounts of Profect for complex formation or to dilute the transfection complexes by 40%. Increase the cell density, increase the amount of protein used for complex formation. We strongly recommend contacting our tech support by email targsys@aol.com

Note: In all instances the transfection complexes should be diluted at least 3X with complete media at the end of the recommended incubation periods for different proteins. In case of longer incubation periods please ensure that the cells are well covered with the protein-Profect mixes.

Protein of interest	Suggested incubation period
Enzymes	2 hours
Antibodies	5 hours and 24 hours
Histone	3 hours and 12 hours
Low molecular weight proteins.	2-3 hours
Peptides	3 hours

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After the suggested incubation periods, you can wash off the transfection complexes by washing cells extensively (4 times with DMEM and assessing protein delivery without fixing the cells). Alternatively, you can aspirate the transfection complexes at the end of the suggested incubation period and add complete media and wait longer to assess effects of protein delivery on the cells. **NOTE**: We recommend using Serum-Free, high glucose DMEM in place of OptiMem 1 as we have observed that it increases cell survival. DMEM can also be used as complexing medium for other applications.

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