NEUROMICS

pro-Fect ™ Transfection Reagent-Frequently Asked Questions

1. What is the pro-Fect Protein Delivery Reagent?

The pro-Fect Protein Delivery Reagent is a novel, cationic lipid designed for the efficient delivery of bioactive molecules, such as proteins, peptides or antibodies, into a broad range of cell types.

2. How does the pro-Fect Reagent work?

When the pro-Fect reagent is combined with a protein in solution, it reacts quickly and interacts non-covalently with the protein, creating a protective vehicle for immediate delivery into cells. The pro-Fect/protein complexes are added onto cells growing in culture, where the complexes can attach to cell's negatively charged plasma membranes. The complexes then fuse directly with the plasma membrane, delivering the captured protein into the cell's cytoplasm, or the complexes are endocytosed by the cells and then fuse with endosomes, releasing the pro-Fect captured protein into the cytoplasm. Delivery of functional proteins or peptides with the pro-Fect reagent is simple, robust, and efficient, requiring only a 4-hour incubation for most cell types.

3. What proteins, peptides, and other molecules can be delivered with pro-Fect Reagent?

A diverse range of proteins and other bioactive molecules has been delivered using the pro-Fect reagent. The molecules delivered in our laboratory include: various peptides, β-galactosidase, FITC-labeled antibodies, high and low molecular weight dextran-sulfate, phycoerythrin-BSA, caspase-3, caspase-8, and granzyme B.

4. What is the largest protein that can be delivered with the Pro-Fect Reagent?

While no upper limit has been established, the largest protein that we have delivered with Pro-Fect was 240 KD (phycoerythrin-BSA).

6. What cell types have been successfully transfected using the pro-Fect Reagent?

In our laboratory, cell types which have been effectively transfected with the pro-Fect reagent include: HeLa-S3, BHK-21, 293, CHO-K1, NIH 3T3, CV-1, B16- F0, COS-1, K562, COS-7, Jurkat, Ki-Ras 267β1, HepG2, MDCK, HeLa, and P19.

7. Do proteins delivered with Pro-Fect Reagent remain functionally active after delivery?

Yes, our data has shown that proteins do retain their biological activity after delivery with pro-Fect reagent. For example, caspase 3, caspase 8, and granzyme B retain the ability to induce apoptosis after pro-Fect-mediated delivery. Additionally, antibodies have retained their target specificity after delivery.

8. When delivering proteins, peptides, and other molecules with pro-Fect Reagent, how pure do they have to be?

Generally, the purer the protein or peptide is, the better. However, the required level of purity depends on the molecule being studied, the contaminants, and the purpose of the study. Because our customers are usually most familiar with these factors, they can usually best determine how pure their molecule of interest must be for their particular application.

9. Has pro-Fect been used to deliver proteins into primary cells?

Yes. We have delivered granzyme B into AML (acute myelocytic leukemia) cells. The delivery efficiency with this protein/cell type was approximately 40% as measured by Annexin V-FITC / propidium iodide apoptosis assay. Also, pro-Fect has been used to successfully deliver proteins into primary human dentritic cells for antigen presentation.

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10. What is the origin of the goat IgG in the FITC-Ab control?
It is whole IgG from non-immunized goats.

12. I want to deliver fluorescent-labeled antibodies into my cells to visualize an intracellular target. How can I do this and distinguish between the bound and free antibodies?
To distinguish between bound and free detection antibodies, try the following suggestions: 1) Use 10 - 50% of the standard antibody amount indicated in the protocol. This will minimize the excess unbound antibody. 2) Look for a punctuate distribution of fluorescence at the expected intracellular location. 3) Use two distinct monoclonal antibodies, each with a different epitope on your target, and each with a distinct fluorophore label (e.g., fluorescein and rhodamine). Look for co-localization of the antibodies at the expected intracellular location. 4) As a negative control, deliver the detection antibodies into cells in which there is no expression of the intracellular target or in which expression has been suppressed.

More Questions? Please call or e-mail:

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