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Protocols For Protein / Peptide Delivery:

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^{o} C. The Profect-P2 reagent can be stored at 4^{o} C or at -20^{o} 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 100 ulof serum-free DMEM

3. Add 3 µl or 5 µl of Profect reagent (mix well before use)

4. Gently mix the transfection complex mixture by flicking the tube.

5. Incubate at room temperature for 20 minutes. Vortex for 15 seconds and dilute to 1 ml with serum free DMEM

6. Remove serum-containing growth media from cells by aspirating, wash cells with serum-free medium and add 1 ml of serum-free medium to each well.

7. Add the transfection complex mixture to cells. Add enough complex to cover cells well for a few hours (300 ul forone well of a 12-well dish, 800 ul per well of a 6-well dish)

8. Return plate to incubator and incubate for 2-5 hours.

9. Add complete media (containing 10% serum) to each well. For a 12 well dish add 1ml media per well, for a 6-well dish add 2 ml media per well

10. Replace media on the following day and continue incubation until assaying. Wash cells with serum-free medium before assaying to remove any untransfected protein. 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 µg protein, and 3 µl Profect Transfection Reagent in 200 µl serum-free medium is added to a well containing cells in a 1 ml volume. Table 1: Protein transfection in different plate formats

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. ß-galactosidase) so that the final protein concentration is about 2 µg total protein complexed with 5 µl 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

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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

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

Peptide Delivery:

Suggested protocol for peptide delivery:

Mix 6.5 µg peptide with 5 µl of the P-2 reagent in 100 µl 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 µl 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 µl of complete media and continue incubation. Wash cells 4 times with serum free media and assay.

TABLE 1: Volumes of plating media for different dishes:

Culture Vessel	Volume of Plating Medium (per well)	Profect transfection complex mixture*
96 well	100 µl	0.2 µl
24 well	200 µl	0.2-2 µl
12 well	0.5 ml	0.4-4 µl
35 mm dish	1 ml	1-10 µl
6 well	1 ml	1-10 µl
60 mm dish	2 ml	4-24 µl

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