NEUROMICS

p-Fect ™ Transfection Reagent-Frequently Asked Questions

1. What are the p-Fect Transfection Reagents?

These Transfection Reagents are polyvalent, cationic lipids that allow high transfection efficiencies to be achieved in a broad range of cell types. Additionally, they provide low cytotoxicity, exceptional stability, and ease of use.

2. How do the Transfection Reagents work?

The positively charged primary amino functional groups in the polar region of the transfection reagents interact with the negatively charged phosphate backbone of the plasmid, DNA or RNA electrostatically. The newly formed DNA/p-Fcet complexes have a net positive charge (when formulated under the recommended conditions), and will bind to the negatively charged plasma membranes of the cells. The complexes are subsequently taken inside the cells via endocyctosis.

3. What are the cell types that work the best?

p-Fect transfection reagents are suitable for working with both adherent (e.g. COS, NIH-3T3, MCF-7) and suspension cultured (e.g. MDCK, K-562) cells, and with both primary and immortalized cells.

4. What is the difference between the original DNA Diluent and the DNA Diluent B?

The DNA Diluent and New DNA Diluent B each contain different proprietary components that enhance transfection efficiency in different cell types. Both DNA diluents are supplied with the p-Fect lipid to allow customers to achieve the highest transfection efficiencies in the broadest range of cell. Here's a table to use with 16 commonly transfected cells. If the cells under study are not among those listed, we recommend starting with the new DNA Diluent B.

Cell Types and Suggested Diluents

Cell Types	DNA Diluent	DNA Diluent B	Serum
HeLa-S3	★	★★	○
HeLa	★	★★	○
COS-1	★	★	●
COS-7	★	★	●
Hep-G2	★	★	●
NIH-3T3	★	★	●
MDCK	★	★★	○
K-562	★	★★	○
CV-1	★	★	●
B16-F0	★	★	●
293	★	★	●
BHK-21	★	★	●
CHO-K1	★▲	★▲	●
PC-12	★	NR	●
P19	★	★	●
HUVEC-C	★	★	●
Jurkat	NR		

LEGEND:

★ Works well.
★★ Works better.
◆ We recommend using the original GenePORTER™ reagent
○ Works well without serum.
● Works well with serum (can also be used without serum)
▲ For CH0-K1 cells highest level of expression are obtained without serum during the first hours of transfection
NR Not recommended.

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5. How is one transfection reaction defined?
One transfection reaction is defined as delivery of 2 ug of DNA in one well of a 6-well plate or in a 35mm dish of cells. Please note that p-Fect Reagent transfection conditions are not based on the surface area or the volume of the culture dish, but rather the number of cells plated. Please refer to the protocols accompanying the product.

6. How soon after the onset of transfection can I assay for gene expression?
Typically, assaying for reporter gene activity may be performed 24-72 hours after the onset of transfection. However, with very slow dividing cells, it is recommended to wait for at least one cell division before assaying for the transgene product.

7. How stable are the reagents before and after hydration?
For maximum stability, store all components of the t kit at 4 ^{o}C upon receipt. If stored properly, the dried p-Fcet reagent is stable for 12 months, and the hydrated reagent is stable for 6 months. The Hydration Buffer and DNA Diluent are stable for 12 months.

8. Why isn't there anything visible in the vial containing the dry p-Fect reagent?
The p-Fect reagents are provided as a dry lipid film to increase long-term stability. The lipid film is essentially invisible to the naked eye, but it is there. Just hydrate the product as instructed in the protocol. (NOTE: In the event that the hydration buffer is accidentally spilled or missing, sterile tissue culture grade water can be substituted).

9. Can the GenePORTER reagents be used for co-transfection of more than one plasmid?
Yes. The p-Fect cells transfection reagents can deliver multiple plasmids into cells. The specified conditions in the protocols are for total DNA quantity. Thus, it does not make any difference whether 1ug of DNA consists of 1 or 10 plasmids.

10. What are the precipitates that settled on top of the cells after adding the DNA/p-Fect Lipoplexes, which are visible under light microscopy?
After adding the DNA/p-Fect complex to the cells, there may be grainy sand-like precipitates settled on the tops of the cells. This is a common observation among all lipid-based transfection reagents. "Precipitates" on top of the cells are the actual DNA/p-Fect complexes attached to the cell surface via electrostatic interaction. The uptake of the desired DNA could not be achieved without the complexes coming into direct contact with cell surface.

23. In what buffer and at what concentration should my DNA be suspended prior to diluting it with serum-free media (or DNA diluents for p-Fect)?
Your DNA can be suspended in TE buffer or purified water. A DNA concentration of at least 0.1 mg/ml works well for most reaction sizes.

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