

Calcium Phosphate Transfection Kit: *Results*

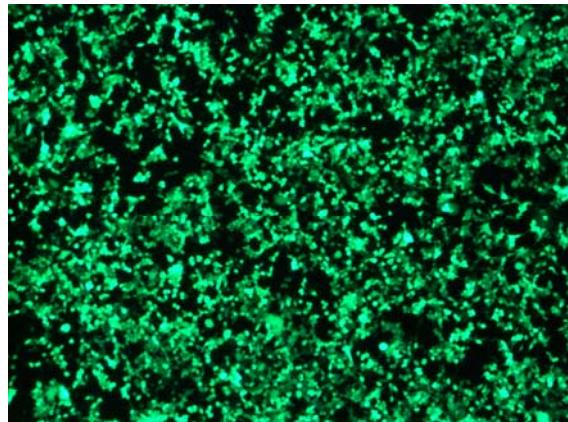
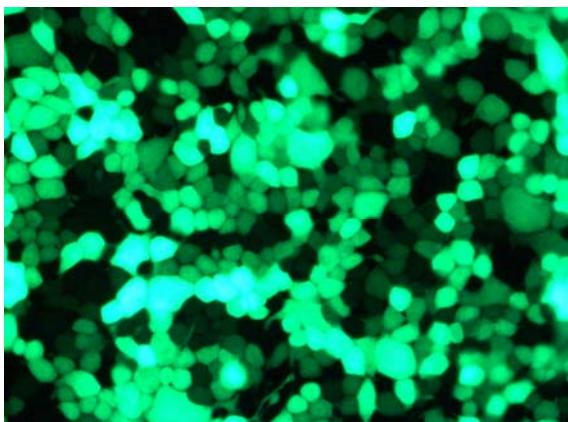
The calcium phosphate transfection method was first described by Graham and van der Ebb in 1973. This method was adapted by several other teams in order to reach higher transfection efficiencies. Calcium phosphate forms a fine precipitate by interacting with DNA allowing the formation of small complexes which are internalized into mammalian cells and transfect them. Although this procedure can be routinely used to transfect a wide variety of cell lines, it gives superior results with the very popular HEK 293 cell line. However, laborious optimizations are usually required to obtain the best of this method. We have modified and optimized it in order to provide you with a very reproducible and efficient Calcium Phosphate Transfection Kit. It will allow reaching between 95 and 100 % HEK 293 transfected cells in your routine experiments. It is ideal for high protein expression and for virus production (higher titer).

Main features are:

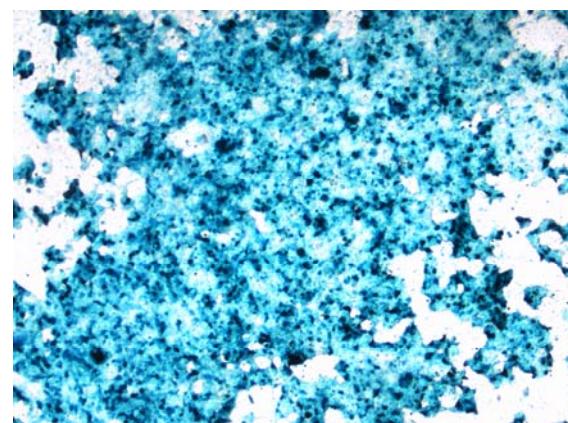
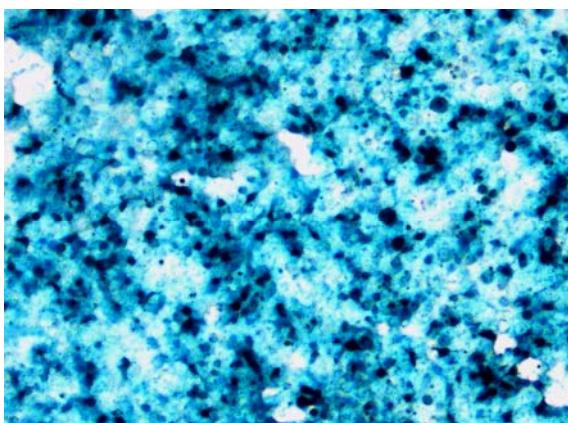
1. Compaction of DNA in nanoparticles efficiently internalized by cells
2. Protection of nucleic acids against nucleases degradation
3. Modified and optimized to reach higher transfection level
4. Ready to use and very reproducible.

The **Calcium Phosphate Transfection Kit** was optimized to be used with plasmid DNA.

HEK 293 cells transfected with the Calcium Phosphate Kit

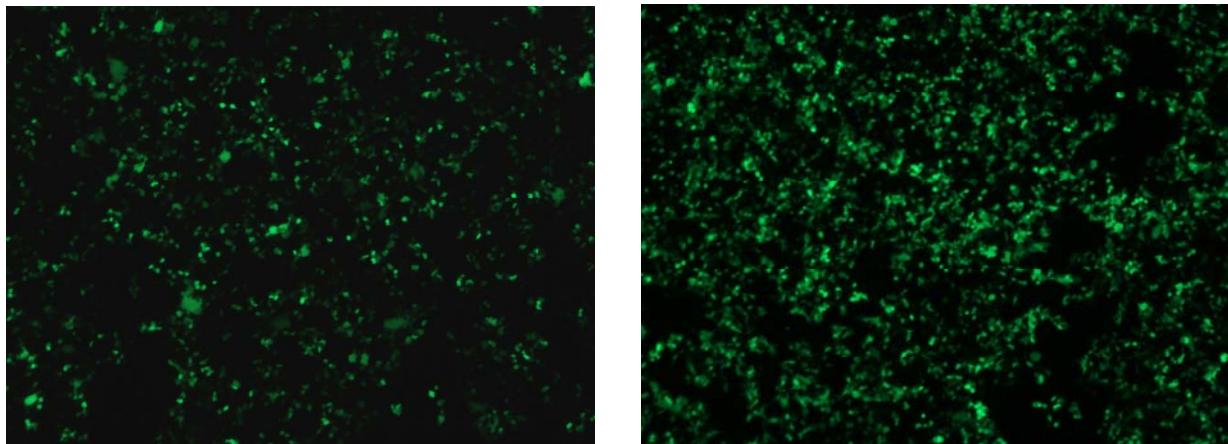


HEK 293 cells were prepared and transfected in 24-well plates, as described in the Calcium Phosphate Transfection Kit protocol, with 1 µg / well of pEGFP plasmid. Transfection efficiency was monitored by fluorescence microscopy 24 h post-transfection.



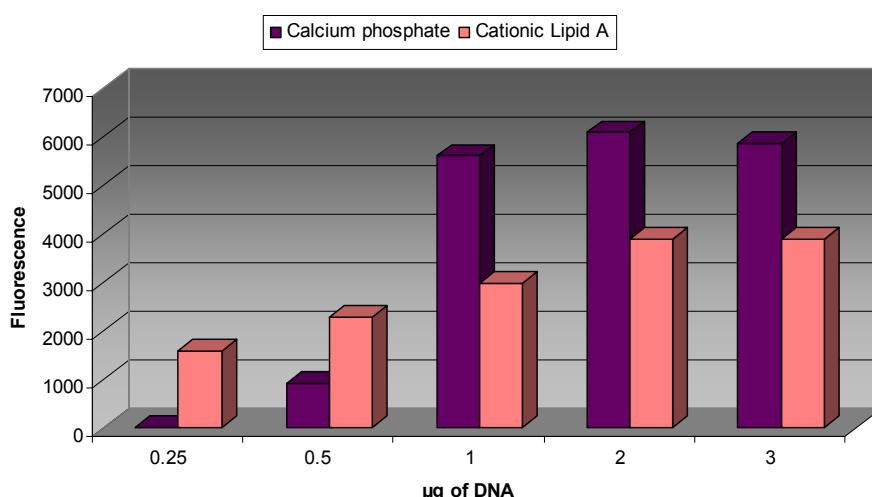
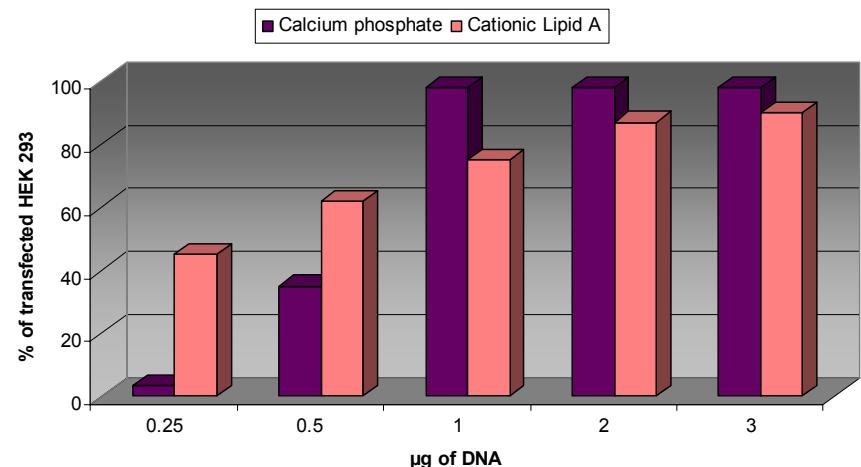
HEK 293 cells were prepared and transfected in 24-well plates, as described in the Calcium Phosphate Transfection Kit protocol, with 1 µg / well of pLACZ plasmid. Transfection efficiency was monitored 24 h post-transfection by quantifying the β -galactosidase activity with X-Gal.

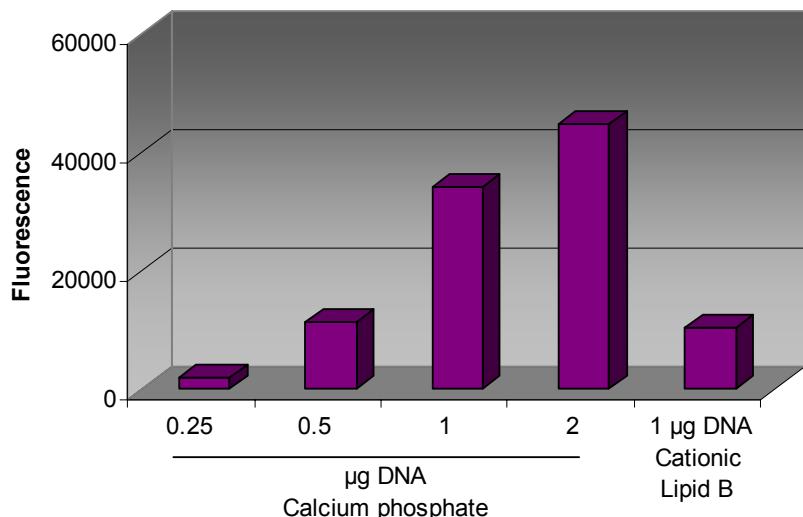
Comparison of transfection efficiency



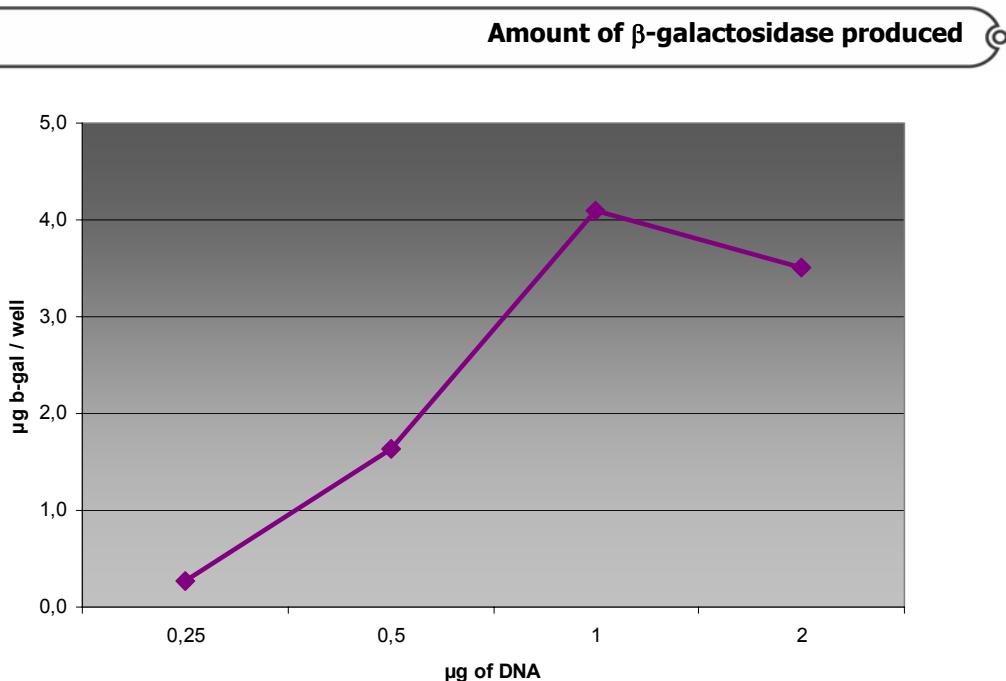
HEK 293 cells transfected with 1 μ g of pEGFP plasmid and a well-known cationic lipid (left) or the Calcium Phosphate Transfection Kit (right). Fluorescence was monitored 24 h post-transfection by fluorescence microscopy and by cytofluorimetry. The number of counted fluorescent cells with the cationic lipid and the calcium phosphate kit was 80% and 98% respectively. The amount of GFP expressed was 100 Relative Unit (RU) with the lipids and 350 RU with the calcium phosphate kit. Pictures were acquired by using the same settings for both experiments. Quantitative data were obtained by cytofluorimetry.

DNA Dose Response with the Calcium Phosphate Transfection Kit





HEK 293 cells were prepared and transfected in 24-well plates, as described in the Calcium Phosphate Transfection Kit protocol, with 0.25 to 3 μ g / well of pEGFP plasmid. Transfection experiments with cationic lipids were performed as instructed by the manufacturer. Transfection efficiency was monitored by cytofluorimetry 24 h post-transfection.

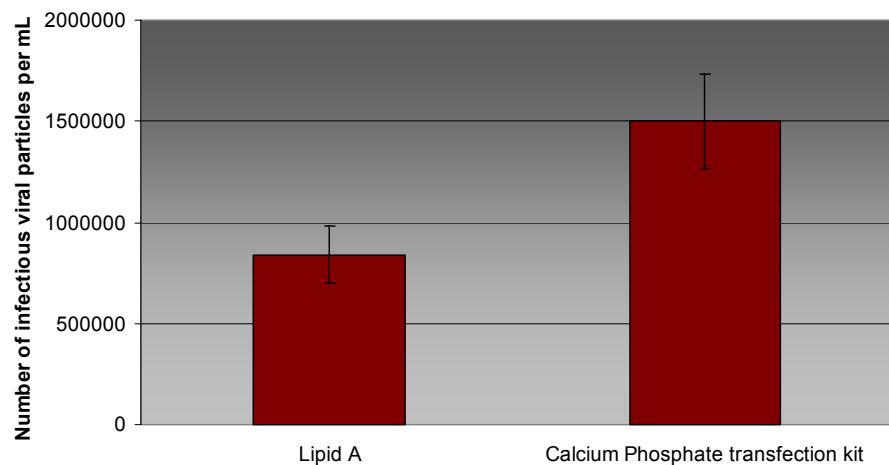


HEK 293 cells were prepared and transfected in 24-well plates with several amount of a pLACZ plasmid encoding β -Galactosidase. The amount of β -galactosidase produced per well was determined by ONPG assay.

Type of cell culture vessel	Amount of protein produced / well or dish
10 cm	140 μ g
24-well	4 μ g
96-well	0.5 μ g

Virus production with the Calcium Phosphate Kit

The full-length molecular HIV-1 (clone LAI) encoding vector (8 μ g) was transfected with an empty vector (12 μ g) in a 100 mm dish with phosphate calcium method. As a control the same amount of the full-length molecular HIV-1 clone LAI encoding vector (8 μ g) was transfected with a competitor's reagent as indicated by the manufacturer instruction manual. Viruses were collected during 48 h and viral titers were determined using HeLa-CD4 beta-galactosidase cells (MAGI assay).



Other cells transfected with the Calcium Phosphate Transfection Kit

The Calcium Phosphate Transfection Kit can be used with numerous cell types but efficiency is highly dependant on the cell type.