Transfection of human HEK 293F cells with METAFECTENE PRO.

Transfection protocol.

HEK 293F cells were plated in 6 well plates (3.5 x 105 cells/well) in 2.5 ml D-MEM medium supplemented with 10% FBS and 1% Penicillin/Streptomycin 24 hours prior to transfection. Cell confluence at transfection was 80-90 %.
Cells were incubated with 2 ml of OPTI-MEM at 37° C for 20 min just before the transfection.

pIRES2-EGFP plasmid DNA was complexed with 250 µl of OPTI-MEM in one tube and METAFECTENE-PRO was complexed with 250 µl of OPTI-MEM in another tube. Solutions were left for 5 min, after which they were combined and left for another 20 min at room temperature.

Cells were transfected with pIRES2-EGFP plasmid DNA:METAFECTENE-PRO in the ratios outlined in Table 1.

Table 1. Ratios of DNA:METAFECTENE-PRO for transfection of HEK 293F cells.

pIRES2-EGFP plasmid DNA (µg)	METAFECTENE-PRO (µl)	Ratios of DNA:METAFECTENE-PRO
1	2	1:2
1	4	1:4
2	4	1:2
2	8	1:4

Transfection mixture was added dropwise to the cells incubated with OPTI-MEM and cells were left in the incubator (37ºC, 5% CO_2) for 5 hours. After which, the transfection medium was removed from the cells and replaced with 2.5 ml of D-MEM supplemented with 10% FBS and 1% Penicillin/Streptomycin.

Evaluating transfection efficiency.

Transfection efficiency was estimated by calculating percentage of cells that displayed green fluorescence. Level of dead cells was evaluated. The results are in Table 2.

Table 2. Transfection efficiency with different ratios of DNA:METAFECTENE-PRO in HEK 293F cells.

pIRES2-EGFP plasmid DNA (µg)	METAFECTENE-PRO (µl)	Ratios of DNA:METAFECTENE-PRO	Transfection efficiency	Levels of dead (detached) cells
1	2	1:2	10 % (extremely faint green fluorescence)	Very few dead cells
1	4	1:4	80% (very faint fluorescence)	Few dead cells
2	4	1:2	70% (faint fluorescence)	Some dead cells
2	8	1:4	Almost 100% (Very bright fluorescence, well defined cells)	High cell death

Conclusion.

METAFECTENE-PRO is an efficient transfection reagent for HEK 293F cells. Increased concentrations of plasmid DNA and METAFECTENE-PRO provides higher transfection efficiency of HEK 293F cells. Unfortunately, due to the sensitive nature of these cells, increasing concentrations of DNA and this transfection reagent leads to the profound cell death. Ratio of 1:2 (2 µg DNA: 4 µl METAFECTENE-PRO) is the most optimal for the transfection of 80-90% confluent HEK 293F cells. These conditions give 70% transfection efficiency with only very moderate cell death.

Similar results were obtained on transfection of HEK 293F cells with LIPOFECTAMINE 2000 (Invitrogen). The only difference observed was when using ratio of 1:4 (2 µg of DNA:8 µl of LIPOFECTAMINE 2000). Transfection efficiency was 70-80% in contrast to almost 100% with METAFECTENE-PRO, but LIPOFECTAMINE 2000 caused less cell death than METAFECTENE-PRO.