K4 technical note

Dr. Cora Stegmann
Division of Biological Sciences, University of Montana, Missoula, MT, USA

Materials: plasmid pEGFP-C1 (GenBank Accession #: U55763)
HEK293T cells (ATCC® CRL-3216)
DMEM medium supplemented with 10% FBS
Sterile 24well tissue culture plates
Sterile 1.5ml snap cap tubes
OptiMEM (serum-free medium)
K4 transfection system

Optimization of HEK293T transfection:

HEK293T cells were grown in DMEM supplemented with 10% FBS, glutamine, penicillin and streptomycin to near confluency. One day prior transfection the cells were trypsinized and seeded onto sterile 24well cell culture plates at a density of 150,000 or 300,000 cells per well in 0.5ml of medium. At the day of transfection, the cells were covering 30% and 70% of the growth area respectively (see Figure 1).

Optimization of the transfection with K4 transfection reagent was carried out as follows:

All reagents were prewarmed to room temperature.
For both cell densities combinations of 2 different concentrations of K4 transfection reagent (1µl or 2µl per well) and plasmid DNA (0.5µg and 1µg per well) were tested.
To prepare the cells for transfection, 5µl of K4 Multiplier were added to each well, the plate was swirled once and the cells were incubated for 30min at 37°C.
During this incubation time the lipoplexes were prepared:
For each well the respective amount (either 1µl or 2µl) of K4 transfection reagent was diluted in 30µl of OptiMEM. The DNA (pEGFP-C1) for each transfection (either 0.5µg or 1µg) was also diluted in 30µl of OptiMEM. The diluted DNA was added to the diluted transfection reagent and mixed by pipetting up and down once.
Incubation for 20min at room temperature.
At the end of both incubation times the lipoplex solutions (60µl total volume) were added dropwise to the wells. The plate was swirled once before further incubation at 37°C.

24 hours post transfection the DNA containing medium was removed and replaced by fresh medium.

2 days post transfection GFP expression was assessed by fluorescence microscopy (Figure 2).

Results and Discussion:

Transfection efficiency reached up to about >90% and was found to be highly dependent on the ratio of DNA to the transfection reagent. A ratio of 1:2 resulted in the highest efficiencies. Furthermore, the higher cell density was favorable. 300,000 cells transfected with 1µg of DNA mixed 1to2 with 2µl of K4 transfection reagent gave the best results.

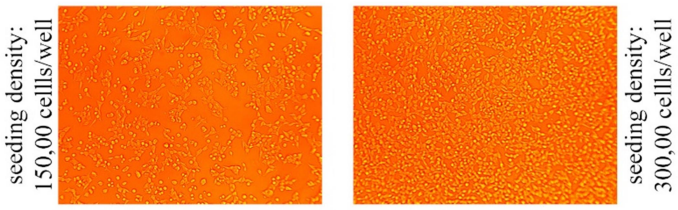

Figure 1: HEK293T cells were seeded at either 150,000 cells or 300,000 cells per well of a 24 well plate in DMEM with 10% FBS. After 24 hours light microscopy images were taken to assess confluency.

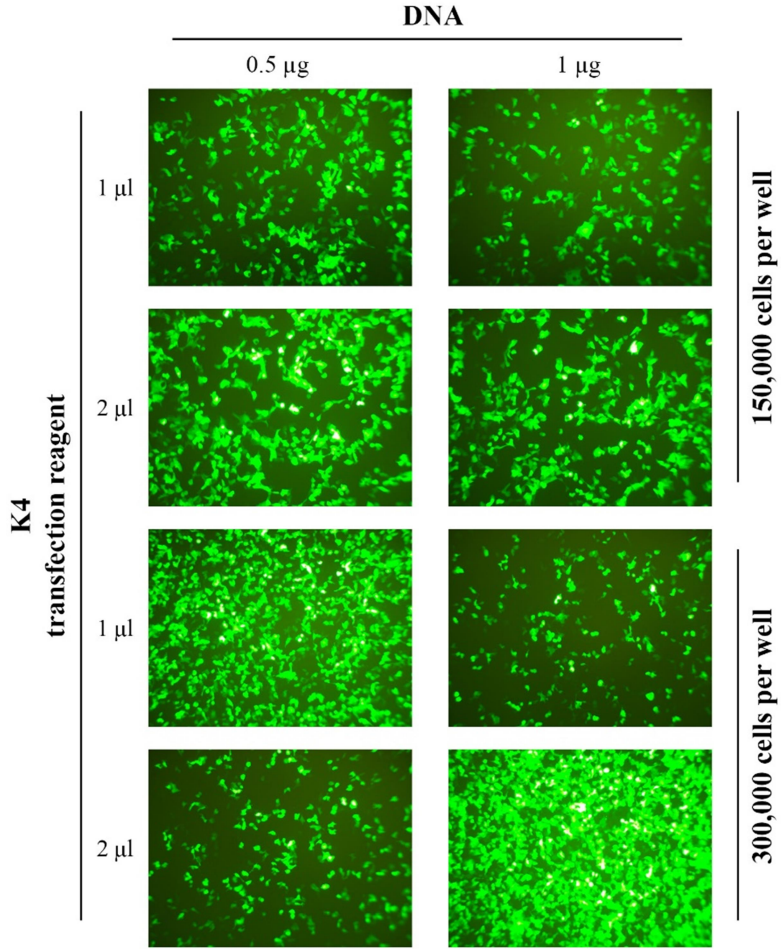

Figure 2: HEK293T cells were seeded at either 150,000 cells or 300,000 cells per well of a 24 well plate in DMEM with 10% FBS. After 24 hours the cells were transfected with a pEGFP plasmid using the K4 transfection reagent. Images of GFP expressing cells were taken at 2 days post transfection.