Transfection of human breast cancer cell line MDA-MB231 with the Biontex K2® Transfection System

Ekin Ece Erkan, N. C. Tolga Emre

Boğaziçi University, Department of Molecular Biology and Genetics, Laboratory of Genome Regulation, Kuzey Park Binasi, 3. Kat, Bebek 34342, Istanbul, TURKEY

Materials and Methods

Cell Culture

Human breast cancer cell line MDA-MB231 was cultured in 2 ml of Dulbecco's modified Eagle's Medium - high glucose (DMEM-Gibco) containing 10% fetal bovine serum albumin (FBS-Gibco) in a humidified atmosphere containing 5% CO_2 at 37°C. It was passaged in 6-well plates (TPP) 1 day before the transfection in order to reach ~70-80% of confluency at the time of transfection.

Transfection

The medium in each well was replaced with fresh DMEM (2ml) containing 40 µl of K2 Multiplier and cells were incubated for 2 hours at 37°C. Adjusting the time to add the transfection mix at the end of 2 hours of incubation period, it was prepared as indicated below:

- Solution A was prepared by adding 2 µg of DNA (green fluorescent protein (GFP) expressing GFP-pIRES2-eGFP plasmid - prepared with Macherey-Nagel endotoxin-free maxi prep kit) to 144 µl of serum-free DMEM in a polypropylene microcentrifuge tube (Eppendorf).

- Solution B was prepared by adding 12 µl of K2 Transfection Reagent to 144 µl of serum-free DMEM in a polypropylene microcentrifuge tube.

- Solution A was added onto Solution B and mixed gently by pipetting up and down.

- The mixture (approximately 300 µl) was incubated for 15 minutes at room temperature and then added to the corresponding wells of a 6-well plate in a drop-wise manner.

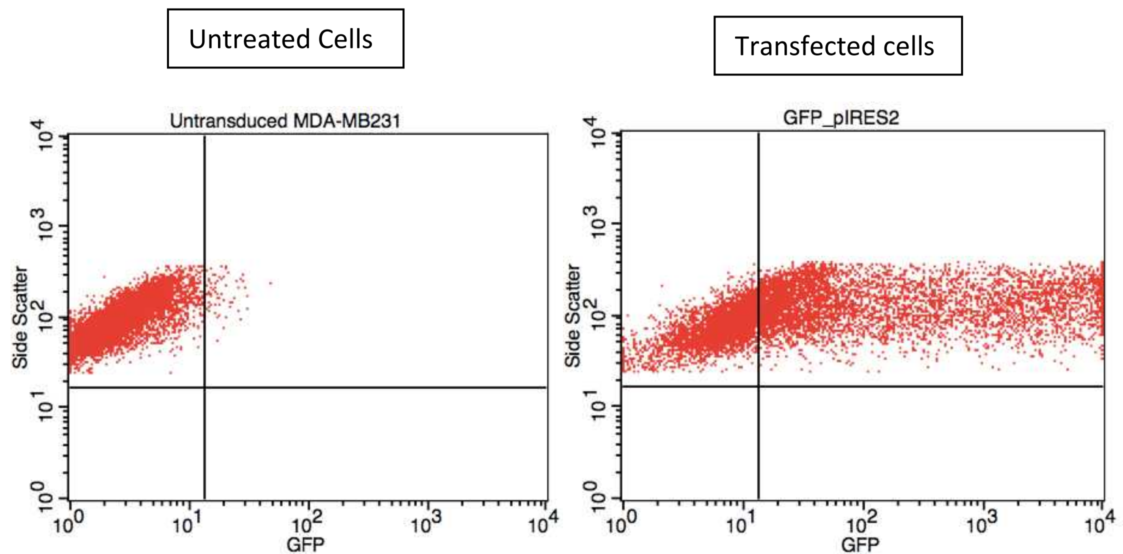
- Transfected cells were incubated at 37°C (5% CO_2) for 6 hours and then the transfection medium was replaced with fresh DMEM containing 10% FBS.

- After 48 hours of incubation at 37°C (5% CO_2), cells were analyzed for GFP signal by flow cytometry (BD FACSCalibur) for transfection efficiency.

Results

Figure below shows flow cytometry results; flow cytometer was used to detect the GFP signal of the cells transfected with the GFP-expressing plasmid, which correlates with the

transfection efficiency. For MDA-MB231 cell line, GFP signal was measured as 59%, when transfected cells were compared with untreated cells. In this cell line, dead cells were observed, about 10% of the transfected cells died after the transfection.



Conclusion

In order to obtain a good transfection, we have tried different transfection conditions by changing the relative amounts of DNA, K2 Transfection Reagent, and K2 Multiplier, or transfection incubation times in other cell lines (data not shown). Based on these pilot results, we were able to achieve high transfection efficiencies with modest toxicity in MDA-MB231 cell line using the Biontex K2® Transfection System.