Transfection of a human melanoma cell line with the Biontex K2® Transfection System

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Materials and Methods

Cell Culture

Human melanoma cell line, SKMEL-28, was cultured in 500 µl 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 24-well plates (TPP) 1 day before in order to reach ~70-80% of confluency at the time of transfection.

Transfection

The medium in each well was replaced with fresh DMEM (500 µl) containing 6 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 0.5 µg of DNA (green fluorescent protein (GFP) expressing pEGFP plasmid - prepared with Macherey-Nagel endotoxin-free maxi prep kit) to 30 µl of serum-free DMEM in a polypropylene microcentrifuge tube (Eppendorf).

- Solution B was prepared by adding 3 µl of K2 Transfection Reagent to 30 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 (60 µl) was incubated for 15 minutes at room temperature and then added to the corresponding wells of a 24-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 a fluorescent microscope and by flow cytometry (BD FACSCalibur) for transfection efficiency.

Results

- In the first two graphs below, GFP intensity of 48h post-transfection cells by flow cytometry are shown. Figure 1A represents the cells without any treatment. Figure 1B depicts the GFP intensity of SKMEL-28 cells transfected with the pEGFP plasmid. Figure 1C shows the GFP signal from transfected cells under fluorescent microscope.

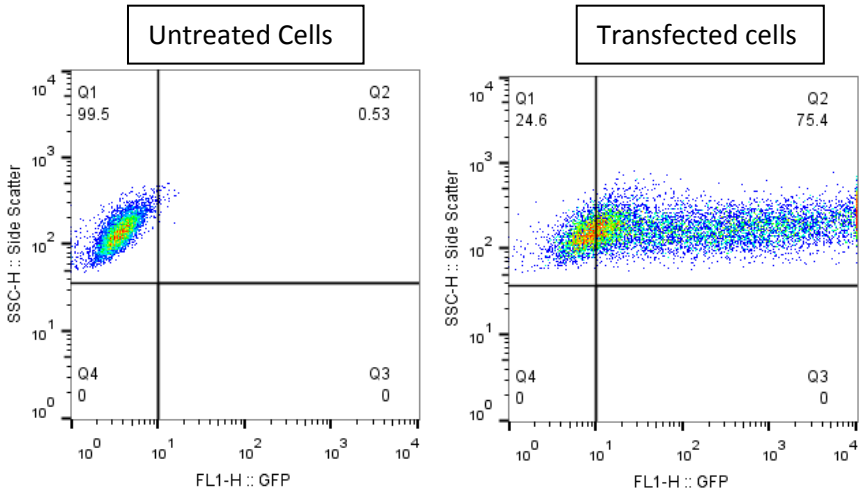

Figure 1A Figure1B

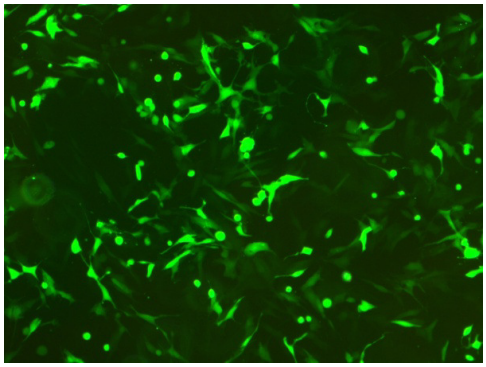

Figure 1C

- Western blotting of whole cell lysates from SKMEL-28 cells transfected with the plasmid containing the coding sequence (CDS) of a protein of interest (pIRES-GFP-CDS) is shown in Figure 2. Transfection protocol was the same as shown above but volumes were adjusted to 60-mm cell culture dish. We got even higher efficiency in this experiment (>95%).

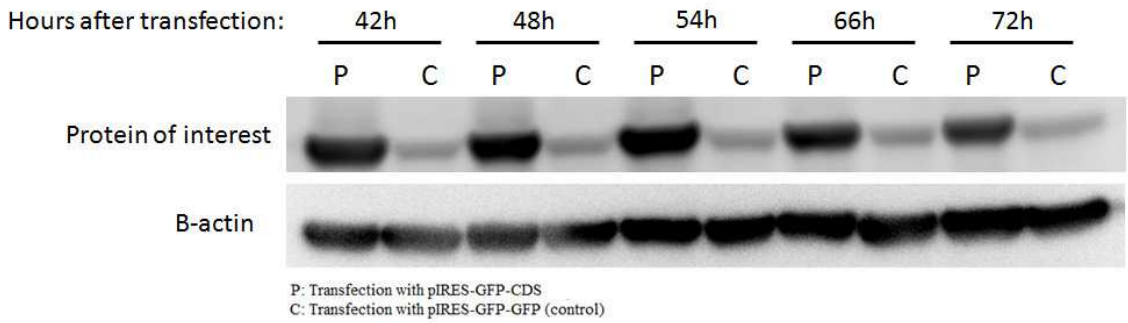

Figure 2

Conclusions

In order to find 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 (data not shown). The protocol presented in this report represents the optimal that we have achieved for the SKMEL28 melanoma cell line. As an additional demonstration of transfection efficiency with Biontex K2®, we also show significantly increased protein levels for an ectopically expressed transgene by western blotting in the same cell line. We have also observed satisfactory transfection efficiencies with other human melanoma cell lines (data not shown). In contrast, our previous transfection trials with other commercial reagents did not yield such satisfactory results in these cell lines. In conclusion, we were able to achieve high transfection efficiencies in human melanoma cell lines using the Biontex K2® Transfection System.