Biontex

DNA-transfection of Human Cervical Cancer Cell Line (HeLa229) and Hematopoietic Stem Cell (CD34+) using “Biontex K2® Transfection System”.

Post-graduate Institute of Medical Education & Research, Chandigarh 160012, INDIA.

Materials and Methods

Cell culture

Human Cervical Cancer Cells (HeLa229) were cultured in 24 well plate (in vitro scientific), in RPMI-1640 with 10% FBS. Transfections were performed when cells reached a confluency of 70-80%.

Hematopoietic Stem Cells were cultured in serum and antibiotic-free DMEM media.

Cell transfection

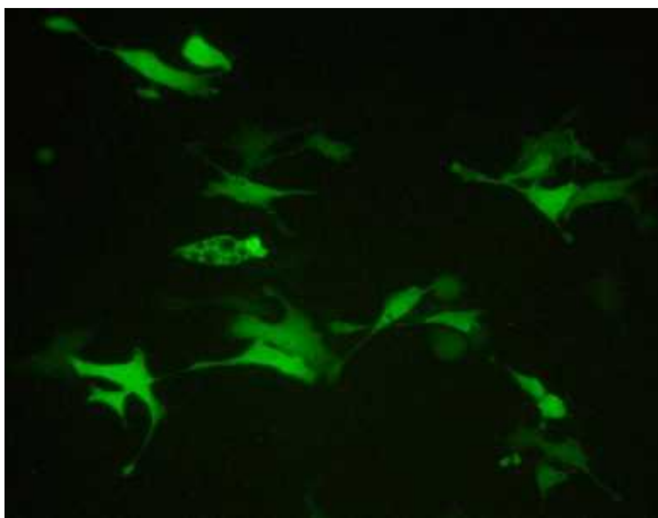
(Please find accurate multiplier & reagent amounts in the table below)

For HeLa229 cells, when cells reached confluency the media was changed with serum-free RPMI-1640. Cells were treated with K2® Multiplier, 2 hours before DNA transfection and mixed by gently swaying the dishes. K2® Transfection Reagent was mixed with serum and antibiotic-free RPMI-1640 and left on room temperature during preparation of the DNA. Plasmid-DNA encoding GFP-miR-2909 (GFP-tagged miR-2909) was mixed with serum and antibiotic-free RPMI-1640. DNA solution was added to the solution containing the K2® Transfection reagent (not the other way around) and mixed by inverting the tubes, followed by 20 minutes incubation at room temperature. Transfection solution was applied to cells by slow drop-wise addition to the medium followed by gently swaying the dishes to achieve mixing. Transfections were incubated at 37˚C and 5% CO_2 for 24 hours. After 24 hours, the RPMI-1640 with 10% FBS was added to cells drop-wise and plate was gently swayed and kept for another 24-48 hours at 37˚C and 5% CO_2. Transfection efficiency was estimated by fluorescence microscopy.

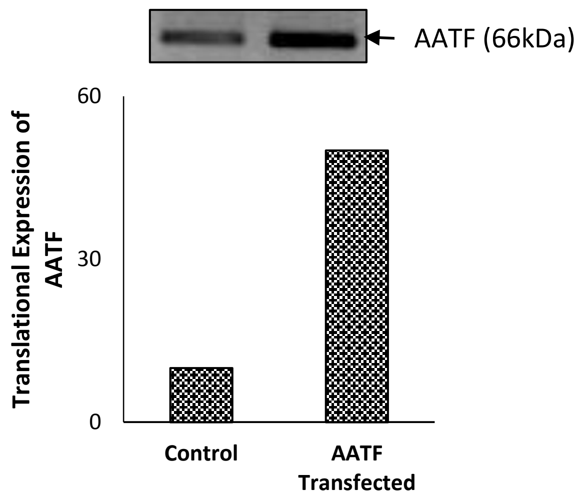
For Hematopoietic Stem Cells, the AATF DNA containing plasmid was transfected using K2 transfection system as described above. Only the media was DMEM rather than RPMI-1640. Protein expression was monitored by immunoblotting for proteins encoded by the transfected plasmids.

Plate	Media	K2® Multiplier	K2® Transfection reagent	Plasmid-DNA
6-well plate	1.5ml	30µl	7.2µl	2400ng
24-well plate	500µl	10µl	2.4µl	800ng
96-well plate	167µl	3.3µl	0.8µl	270ng

Results



GFP-miR-2909 Vector Transfected Hela229 cells



AATF plasmid Transfected Hematopoietic Stem Cells

Conclusions

Fluorescent microscopy showed successful transfection of GFP-miR-2909 with high transfection rate. The cells showed perfect healthy morphology. For the immunoblot, DNA encoding AATF was transfected into Hematopoietic Stem Cells using the K2® transfection system. The blot showed a band of the expected size (~66kDa). Aside from this, it showed a significantly higher band representing the successful transfection of the plasmid DNA. Taken together, data showed high transfection rate (~75-80%) with very negligible cytotoxic effects due to transfection reagent.

In contrast to other transfection reagents we found K2® Transfection system advantageous because:

1. **Efficiency**

   The above mentioned protocol adopted by us ensured transfection efficiency ranging from 75-80% with least toxicity.

2. **For all cell types**

   Even for the stem cells that were not capable of transfection with other transfection reagents, this worked very well. Even the cell morphology and transfection efficiency was perfect after transfection. It would be pertinent to try this transfection reagent in human blood mononuclear cells because transfection efficiency in these cells is highly poor with other commercially available transfection reagents.