Transfection of an immortalized retinal cell line using K2® transfection reagent – Brief Report

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Original purpose of the experiment:

To determine the most optimal K2® formulation for the transfection of A7 retinal astrocytes seeded with different population by evaluating its transfection efficiency and toxicity after 48 hours.

Material:

- K2® Transfection System (K2® Reagent + K2® Multiplier) (Cat#T060, Biontex, Germany)
- gWiz™ GFP (green fluorescence protein) reporter plasmid (Aldevron, Fargo, ND, USA)
- A7 rat astrocytes [1,2]
- DMEM/High Glucose media (supplemented with 10% fetal bovine serum and 1% penicillin streptomycin) (Thermo Fisher Scientific Waltham, MA, USA)
- TrypLE Express reagent (Thermo Fisher Scientific)
- 12- or 24- well tissue-culture treated plates (MatTek Corporation, Ashland, MA, USA)
- MitoTracker® Deep Red FM (Thermo Fisher Scientific)
- CellMask™ Deep Red Plasma Membrane Dye (Thermo Fisher Scientific)

Method:

1. A7 rat astrocyte, an immortalized astrocyte cell line was cultured with DMEM/High Glucose media supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (complete media) in T75 cell culture treated flask until 70% confluency.

2. Cells were trypsinized using TrypLE Express and appropriate number of cells (25,000, 50,000, or 100,000 cells) were seeded into each well of either 12-well (for 50,000 and 100,000 cell seeding density) or 24-well (for 25,000 cell seeding density) tissue culture treated plates supplemented with 1mL of complete media . The plates were incubated in a 37°C incubator with 5% CO_2 overnight prior to transfection.
3. Two hours prior to transfection, 3.75µL of K2® Multiplier were added directly into each well containing cells and the plates were incubated at 37°C.
4. In the meantime, K2® transfection particles were prepared according to manufacturer's protocol. Please refer to Table 1 for specific quantities used.
5. After two hours of K2® Multiplier incubation, the prepared K2® transfection particle was added into each well and incubated at 37°C for 48 hours. The specific dose volume varies between the formulations and seeding density as shown in Table 1.
6. After 48 hours of transfection, cells were trypsinized using TrypLe™ Express and stained with 1mL of 1µM MitoTracker® Deep Red for 30 minutes at 37°C prior to flow cytometry evaluation. Transfection efficiency was determined based on GFP expression and the viability was evaluated based on MitoTracker® fluorescent emission.

Table 1 – Composition of the different K2® formulation

Transfection Agent = K2®		
Plate Format = 12-well		
	Formulation#1	Formulation#2
K2(µL)	2	4
DNA(µg)	1	1
Number of wells	18	18
Tube#1 - Media-DNA (µL)	900	900
Tube#2 – DNA (µL)	36	36
Tube#3- Media-K2 (µL)	900	900
Tube#4- K2 (µL)	36	72
Total Volume (µL)	1872	1908
Volume per well (µL)	104	106

Transfection Agent = K2®		
Plate Format = 24-well		
	Formulation#1	Formulation#2
K2(µL)	2	4
DNA(µg)	0.5	1
Number of wells	9	9
Tube#1 - Media-DNA (µL)	270	270
Tube#2 – DNA (µL)	9	18
Tube#3- Media-K2 (µL)	270	270
Tube#4- K2 (µL)	18	36
Total Volume (µL)	567	594
Volume per well (µL)	63	66

Results:

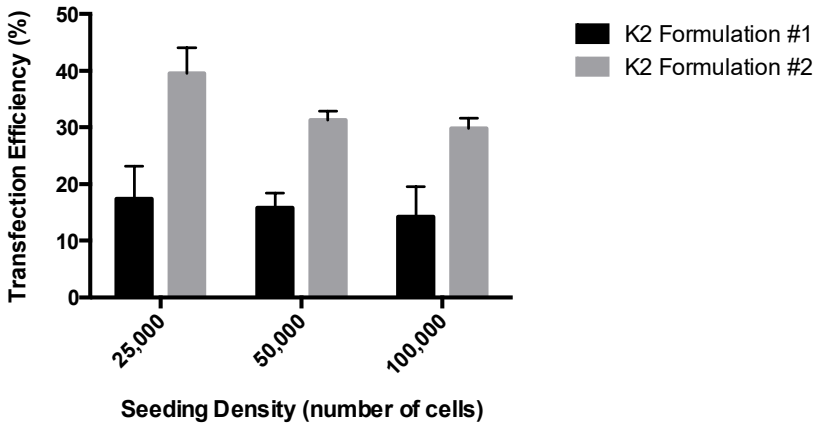

Figure 2 – Comparison between the transfection efficiency of A7 retinal astrocytes transfected with two different formulation ratios of K2® transfection reagents after 48 hours.

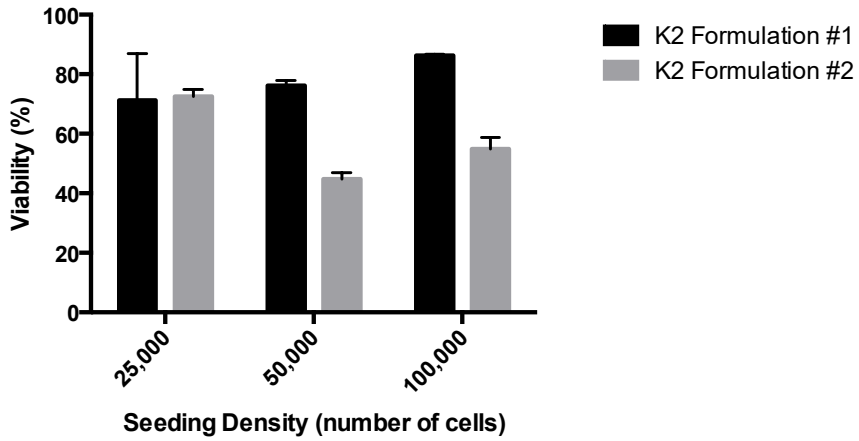

Figure 3 – Comparison between the viability of A7 retinal astrocytes transfected with two different formulation ratios of K2® transfection reagent after 48 hours.

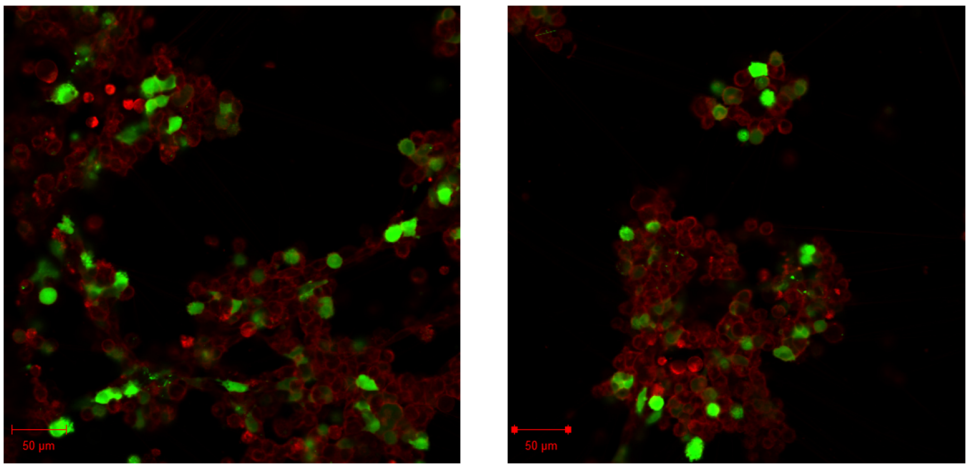

Figure 4 – Confocal microscopic images of A7 astrocytes transfected with K2 transfection reagent (Formulation 2) after 48 hours. *Green* representing GFP expression and *red* representing the cellular membrane stained with CellMask™ Deep Red Plasma Membrane dye.

Conclusion:

K2® transfection system has demonstrated to be effective in the transfection of A7 cells and is currently being used in downstream experiments.

References

[1] H.M. Geller, M. Dubois-Dalcq, Antigenic and functional characterization of a rat central nervous system-derived cell line immortalized by a retroviral vector. J Cell Biol. 107 (1988) 1977-1986.
[2] M. Noble, K. Murray, Purified astrocytes promote the in vitro division of a bipotential glial progenitor cell. EMBO J. 3 (1984) 2243-2247.
[3] Chen DW and Foldvari M. *In vitro* bioassay model for screening non-viral neurotrophic factor gene delivery systems for glaucoma treatment. Drug Deliv. Transl. Res. (2016) doi:10.1007/s13346-016-0324-9