DNA-transfection of primary neonatal rat ventricular cardiomyocytes cells using “Biontex K2® Transfection System”

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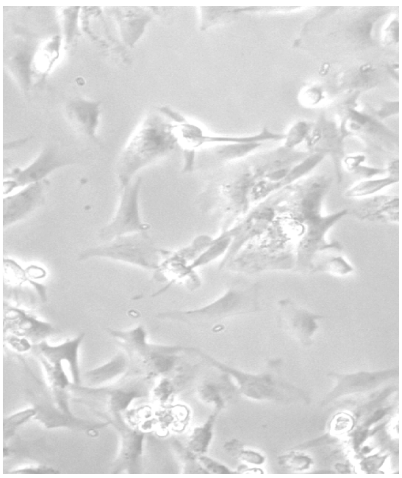
Materials and Methods

1. 500,000 primary neonatal rat ventricular cardiomyocytes cells were plated in each well of a 12-well dish in 1 ml of Dulbecco’s modified eagle medium (DMEM) supplemented with 10% fetal calf serum and 10,000 U/ml-10mg/ml Penicillin-Streptomycin.
2. Cells were incubated for 48h at 37°C in a CO_2 incubator.
3. Cells in 800 µl medium were treated with 12 µl of K2® Multiplier 2 hours before adding the lipoplex. For this, K2® Multiplier was dripped slowly onto the medium and mixed by gently swaying the dishes.
4. For each well of a 12-well dish there were prepared:
Solution A: 1 µg of plasmid-DNA encoding either for GRK2 mutant or control plasmid (empty pCEFL vector) was mixed with 80 µl medium without serum.
Solution B: 2 µl of K2® Transfection reagent was added to 80 µl medium without serum.
Solution A was added to the solution B (not the other way around) and mixed by inverting the tubes, followed by 20 minutes incubation at room temperature. Transfection mix was applied to cells by slow dropwise addition to the medium followed by gently swaying the dishes to achieve mixing. Transfected cells were incubated at 37C and 5% CO_2 for 48 hours and then total cell lysates were analyzed by western blot to evaluate GRK2 transfection.
5. 24 hours after transfection media was replaced with fresh media.
6. 48 hours after transfection cells were lysed with Laemmli Buffer for western blot evaluation.

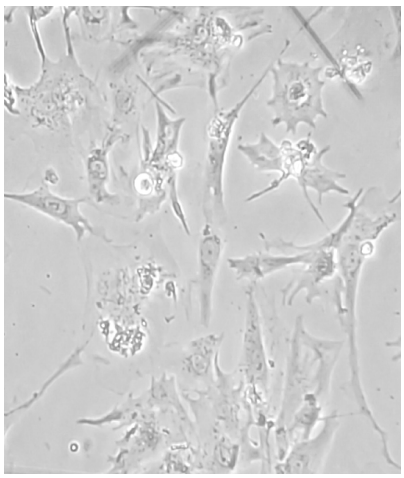
<u>Evaluation of protein expression</u>
Effective transfection was evaluated by western blot assay using anti GRK2 antibody and anti α-tubulin as loading control.

Results

A

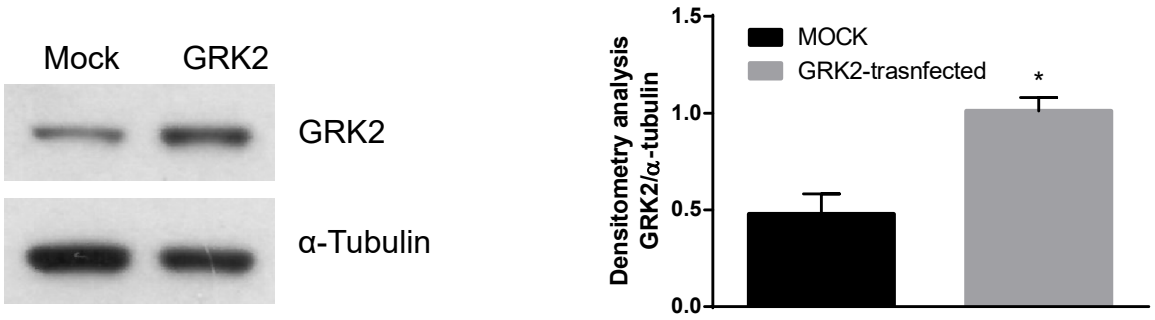


B



Representative image of untransfected (A) and transfected (B) primary cardiomyocytes neonatal rat cells (40x).

Overexpression of GRK2.



Western blot showing increased expression of GRK2 after transfection on primary neonatal rat ventricular cardiomyocytes. Densitrometic analysis was performed using Image J software. (*) $p<0.05$.

Conclusions.

Our results show that primary neonatal rat ventricular cardiomyocytes are efficiently transfected with **Biontex K2® Transfection System** reagent.