DNA Transfection for Luciferase Assay in Human Alevolar Adenocarcinoma Cells (A549) using Biontex K2 ® Transfection System

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Purpose of Experiment:

We tested two different transfection reagents (Biontex K2® v.s. Lipofectamine 2000®) to determine which reagent has higher transfection efficiency and lower cell toxicity. We used luciferase assay to assess transfection efficiency and evaluate cytotoxicity using microscopy.

Material and Method:

Cell Culture:

A day before DNA transfection, 1×10^5A549 cells were cultured in 24-well plate with RPMI-1640 media containing 10% FBS without antibiotics.

DNA Transfection:

1. **On the day of DNA transfection, we added 5ul K-2 ® multiplier in each well for K-2 group and cell were incubated at 37°C, 5%CO_2 for two hours.**
2. **Plasmids were prepared for luciferase assay in K-2 and Lipofectamine-2000 groups (duplicate well for each group) as following:**
   - **Tube 1A: pcDNA3.1 EV 225ng (2uL) + SPB 225ng (2uL) + Renilla 50ng (2uL) + 54uL opti-MEM**
     Tube 2A: TTF-1 WT 225ng (2uL) + SPB 225ng (2uL) + Renilla 50ng (2uL) + 54uL opti-MEM
   - **Tube 1B: 5uL of either K-2 or Lipofectamine-2000 + 55uL opti-MEM**
     Tube 2B: 5uL of either K-2 or Lipofectamine-2000 + 55uL opti-MEM
3. **Then mix tube A and Tube B by pipetting up and down few times. Take 60uL of combined transfection solution and add into each well of each group, then incubate cells at 37°C, 5%CO_2 for 24 hours.**
4. **After 24 hours, remove medium containing transfection solution in each well and replace with fresh medium (RPMI-1640 media containing 10% FBS without antibiotics) and then incubate cells at 37°C, 5%CO_2 for another 24 hours .**
5. **After incubation, we use firefly luciferase assay kit (Biotium, # 30005-2) to assess transfection efficiency by determining promoter activity of Surfactant protein B trans-activated by TTF-1 (NKX2.1).**
6. **Luciferase activity is recorded by GLOMAX 96 microplate Luminometer (Promega).**

Result:

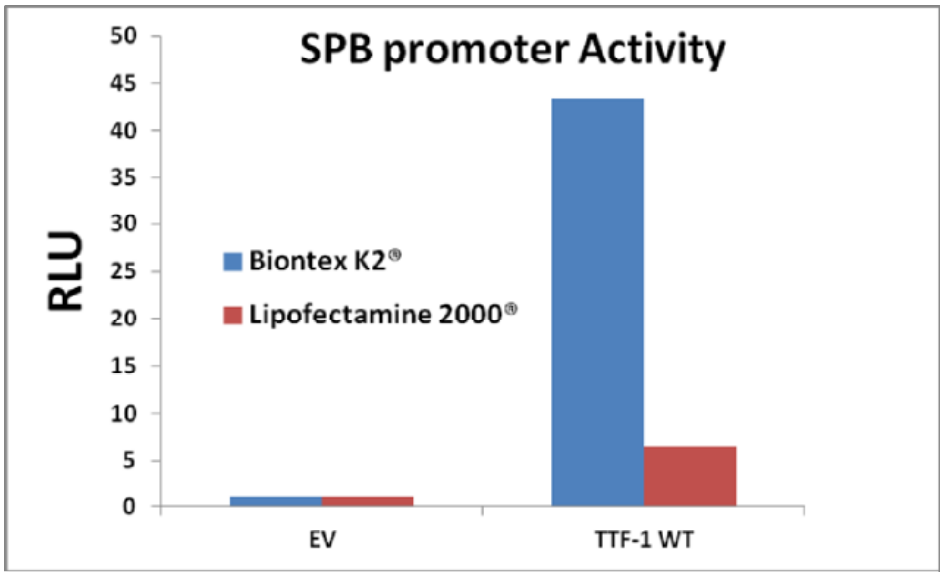

Fig.1 Surfactant Protein B (SPB) promoter Activity. SPB 1kb promoter region and TTF-1 wild-type (WT) plasmid were co-transfected in A549 cells using either Biontex K2® or Lipofectamine 2000® transfection reagent. SPB promoter activity was determined by firefly luciferase assay. Data was collected from duplicate well of each group and normalized by Renilla reporter reading. EV: empty vector control (pcDNA3.1). TTF-1 WT: TTF-1 wild type cDNA cloned into pcDNA3.1.

Conclusion:

We observed that TTF-1 WT transactivated SPB promoter as demonstrated by a 43-fold increase of luciferase activity when we cotransfected TTF-1 WT and SPB promoter reporter using Biontex K2® transfection reagent. On the other hand, there was only a 6-fold increase in the SPB promoter reporter activity when we used Lipofectamine 2000® transfection reagent. Moreover, we also observed less cytotoxicity when we used Biontex K2® transfection reagent compared with the Lipofectamine 2000® transfection reagent. Clearly, the data suggest that Biontex K2® transfection reagent demonstrates a higher transfection efficiency and Biontex K2® is an excellent tool in conducting promoter reporter experiments using the luciferase assay system.