DNA-transfection of COS 7 and MEF cells using Biontex K2 Transfection System in comparison with Invitrogen Lipofectamine Plus Reagent.

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Materials and Methods

Cell cultures

COS 7 and MEF cells were cultured on 100-mm tissue culture dishes (Falcon), in 10 ml of Dulbecco's Modified Eagle's Medium (DMEM) supplemented with L-glutamine, Penicillin+Streptomycin and 10% fetal bovine serum (FBS).
Cells were grown to 70% confluency in transfection test nº1 or to 90% confluency in transfection test nº2. They were transiently transfected with 6 εg of pceflGST-human-oncoDbl construct (8100 bp).

Cell transfection

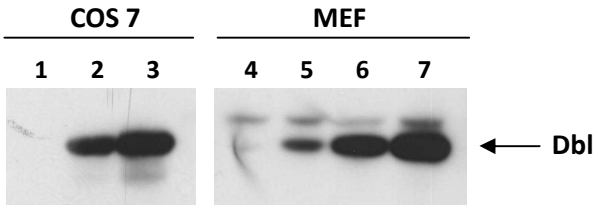
Cells were transfected using Lipofectamine Plus (Invitrogen) as described by the manufacturer, or using Biontex K2 Transfection System in parallel.
In the transfection test nº1 we used 300 εl Multiplier and 70 εl of Transfection Reagent, while in the transfection test nº2 we used 200 εl Multiplier and 30 εl of Transfection Reagent. Both transfection tests were performed as follows:
2 hours incubation of cells with Multiplier
Preparation of Solution A with 750 εl serum-free DMEM and 6 εg DNA
Preparation of Solution B with 750 εl serum-free DMEM and Transfection Reagent
Mix of solution A into solution B and incubation of 20-30 minutes
Addition of mixed solution to cells and 24 hours incubation
Replacement with fresh complete growth medium.

Western blot analysis

24 hours after the medium replacement, cells were lysed on ice with a specific lysis buffer and 100 εg of each lysate were subjected to 6% SDS-PAGE electrophoresis, transferred to PVDF membrane (Millipore) and probed with the polyclonal anti-human Dbl antibody (Santa Cruz Biotechnology).

Results and conclusions

Cells from transfection test nº1 showed a certain suffering, especially COS 7, while cells from transfection test nº2 were comparable to cells from Lipofectamine Plus transfection. The Dbl protein expression was surely stronger with K2 Transfection System than with Lipofectamine Plus Reagent, and better with a higher cell confluency combined to a lower amount of K2 reagents (see the figure).



Lane 1: untransfected COS 7 cells
Lane 2: COS 7 cells transfected with Lipofectamine Plus Reagent
Lane 3: COS 7 cells transfected with K2 ï Transfection test nº2
Lane 4: untransfected MEF cells
Lane 5: MEF cells transfected with Lipofectamine Plus Reagent
Lane 6: MEF cells transfected with K2 ï Transfection test nº1
Lane 7: MEF cells transfected with K2 ï Transfection test nº2