

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

Marzia Ognibene, Molecular Biology Laboratory, G. Gaslini Institute, Largo Gaslini 5, 16147 Genova, Italy

### **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 n°1 or to 90% confluency in transfection test n°2. They were transiently transfected with 6 µg of pcefIGST-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 n°1 we used 300 µl Multiplier and 70 µl of Transfection Reagent, while in the transfection test n°2 we used 200 µl Multiplier and 30 µl of Transfection Reagent. Both transfection tests were performed as follows:

2 hours incubation of cells with Multiplier

Preparation of Solution A with 750 µl serum-free DMEM and 6 µg DNA

Preparation of Solution B with 750 µl 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

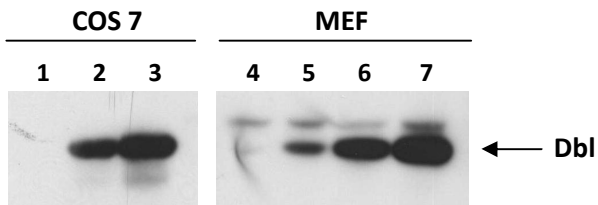
Replacement with fresh complete growth medium.

#### Western blot analysis

24 hours after the medium replacement, cells were lysed on ice with a specific lysis buffer and 100 µg 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 n°1 showed a certain suffering, especially COS 7, while cells from transfection test n°2 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 . Transfection test n°2

Lane 4: untransfected MEF cells

Lane 5: MEF cells transfected with Lipofectamine Plus Reagent

Lane 6: MEF cells transfected with K2 . Transfection test n°1

Lane 7: MEF cells transfected with K2 . Transfection test n°2