Biontex

Silencing of GAPDH gene in human primary dermal fibroblast with K2 Transfection System

Joanna Borkowska, Paulina Kołodziej
Mossakowski Medical Research Centre, Polish Academy of Sciences
5 Pawińskiego Str., 02-106 Warsaw, Poland

Materials

- DMEM, high glucose, GlutaMAX™ Supplement, HEPES (Gibco)
- Fetal Bovine Serum (Sigma)
- PBS
- Trypsin solution
- Sterile Eppendorf tubes
- 75T cell culture flask (BD bioscience)
- 6-well plate (BD bioscience)
- GAPDH siRNA *(Silencer® Select GAPDH Positive Control siRNA)*
- *Silencer® Select Negative Control No. 1 siRNA*
- Transfection reagents:
  K2 transfection reagent
  K2 multiplier

Protocol

1. Primary human fibroblasts were grown in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin in T75 cell culture flask. Cells were trypsinized after 80% confluence and seeded on 6-well plate in density $3x10^3/cm^2$ ($3x10^4$ cells per well in 2ml medium).
2. Next day K2 multiplier (40µl) was added to cells 2h prior transfection. Cells were transfected with GAPDH siRNA or negative control siRNA according to table and following protocol:

Plate: **6-well plate**
Amount of medium per well: **2ml**
K2 multiplier: **40µl, 2h incubation**

siRNA [pm]	DMEM [µl]	K2 [µl]	DMEM [µl]
20	130	3	130
20	130	3	130

3µl K2 transfection reagent was added to 130µl of serum-free cell culture medium (DMEM) in a 1.5 ml Eppendorf centrifuge tube for each siRNA. In a separate tube 20pm siRNA (GAPDH siRNA or negative control, 1µl of 20µM stock) was mixed with 130 µl of serum-free medium. The media containing K2 and siRNA were combined and mixed by pipetting up and down 5 times. The tubes were incubated at room temperature for 20 min for lipid-DNA complexes to form. At the end of the incubation time, the lipid-DNA complex mixtures (260 µl per well) were pipetted onto the cells, mixed by tilting the plates a few times and thereafter incubated at 37°C incubator under 5% CO2.

After 48 hours cells were scrapped for RNA isolation. GAPDH gene expression was analyzed by Real Time PCR.

Results

GAPDH gene expression on mRNA level was 67 fold lower after transfection with GAPDH siRNA and K2 transfection reagent.

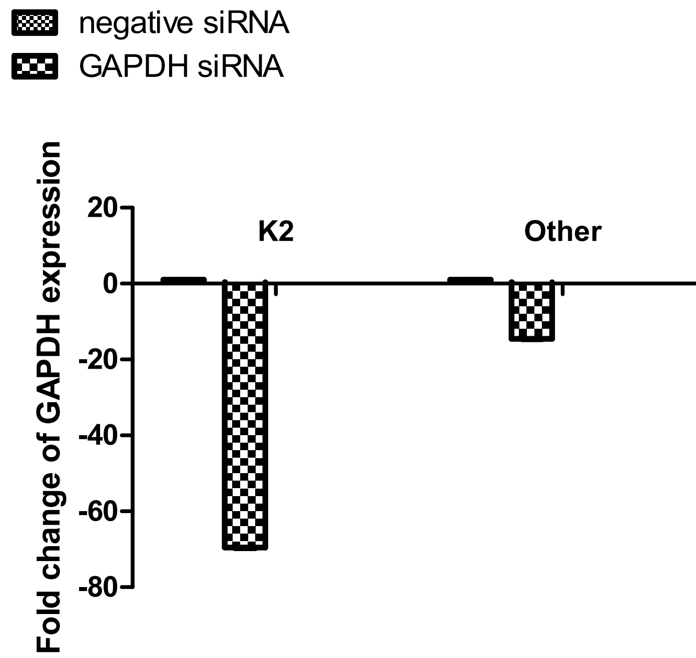

Figure 1. Silencing of GAPDH with siRNA using K2 transfection reagent (K2) or other agent (Other). Graph shows fold expression change of GAPDH mRNA. Control cells were transfected with negative siRNA. B-actine was used as a reference gene.