Biontex

K2 Transfection Technical Note

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Materials:
L6 muscle cells
Sterile 10-cm and 24-well tissue culture plates
GFP::pGL3 and flag-actin::pcDNA3.1 plasmids
Time-lapse microscope
Western blot system

Growth medium:
DMEM 4,5 g Glucose
2 % L-Glutamine
1 % Non Essential Amino Acids
1% Penicillin-Streptomycin
10 % Fetal Calf Serum

Differentiation medium
DMEM 4,5 g Glucose
2 % L-Glutamine
1 % Non Essential Amino Acids
1% Penicillin-Streptomycin
2% Horse Serum

Transfection reagent
K2® -transfection reagent
K2® -multiplier

Transfection of plasmids encoding for GFP or flag-actin into L6 myotubes:

L6 cells were grown using growth medium in 10-cm dishes until 50% confluence and then seeded in 24-well tissue culture plates. At 80-90% confluence, L6 cells were washed with sterile 1X PBS and differentiated in low-serum differentiation medium for 7 days. L6 cells were transfected either at day 0 of differentiation (myoblasts) or at day 5 of differentiation (myotubes). When differentiated, these cells are known to be resistant to transfection using common transfection reagents.

Cell lysis: 48h post transfection

Transfection of plasmids encoding either for GFP or flag-actin:
Transfection was performed using either a 1:4 or a 1:5 plasmid:K2®-transfectant reagent ratio and with 0, 2.5 or 5 µl of K2® -multiplier
The differentiation medium was removed 2 hours prior to transfection and 415 µl of fresh medium containing either 0, 2.5 or 5 µl of K2®-multiplier were added in each well.

1:4 plasmid:K2®-transfectant reagent ratio:

Solution A

- 20 µg of plasmid (10 µl) were added to 1000 µl of DMEM in a sterile Eppendorf

Solution B

- 18 µl of K2® -transfection reagent was added to 225 µl of DMEM in a sterile Eppendorf

1:5 plasmid:K2®-transfectant reagent ratio:

Solution A

- 20 µg of plasmid (10 µl) were added to 1000 µl of DMEM in a sterile Eppendorf

Solution B

- 22.5 µl of K2® -transfection reagent was added to 225 µl of DMEM in a sterile Eppendorf

375 µl of solution A was added to 375 µl of solution B and the mixture was gently mixed by pipetting up and down. The solution was incubated at room temperature for 20 minutes.

- Cells transfected with GFP were observed using a time lapse microscope
- Cells transfected with pcDNA3.1::flag-actin were analyzed by Western blot using the anti flag antibody.

Conditions:

Different conditions were tested:

- L6 myoblasts or myotubes
- Ratio DNA/K2: 1:4 or 1:5
- DNA amounts: 0.3 or 0.5 µg per well

Two replicates per conditions were realized.

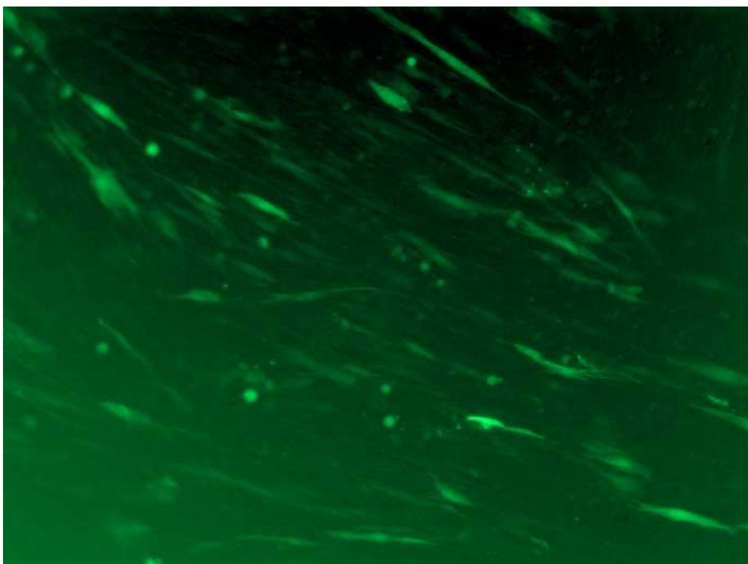
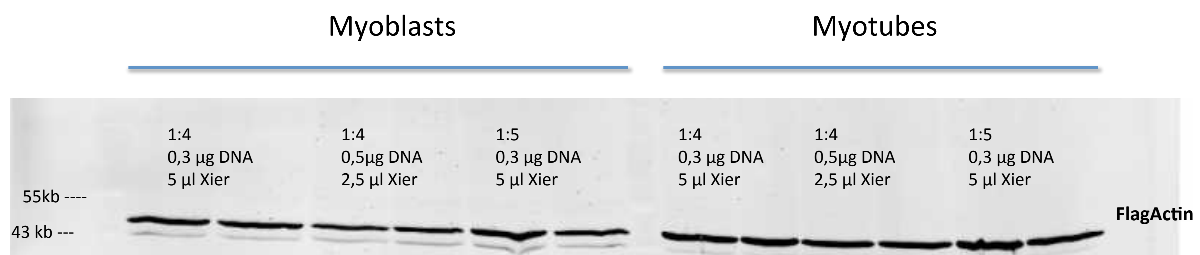


Fig. 1: L6 myotubes transfected with GFP were observed using a time-lapse microscope. The cells were observed daily for 9 days. The expression of GFP peaked at day 5 of transfection with around 50 % of positive green cells. Few if any variation was observed between the different conditions used [the 1:5 DNA:K2 ratio (50 µg DNA) at day 5 of transfection is shown].

Fig. 2: The expression of flag-actin (see below) was addressed by Western blot anti-flag at day 3 of transfection. L6 myoblasts and myotubes were scrapped at day 3 of transfection (i.e. the peak of expression of flag-actin) and lyzed by sonication (30 seconds at maximum power) in lysis buffer [(50 mM Tris. pH 7.5; 5 mM EDTA; 1 mM PMSF; 10 mM NEM; 1% Triton X-100; and 1% anti protease cocktail (Sigma)]. Lyzed cells were then centrifuged at 15000g, 10 minutes at 4°C. Thirty µg of the supernatant was loaded onto a 10% SDS-PAGE and flag-actin was detected by Western blotting using the anti-flag M2 antibody (1/1000, Sigma) and fluorescent anti mouse antibody 800CW (1/5000, Eurobio). The signal was detected using a LI-COR-Odissey Imager (LI-COR Biosciences). Flag-actin was expressed in both myoblasts and myotubes at similar levels whatever the conditions used, even though the use of 5 µl of multiplier seemed to increase the efficiency (Xier, multiplier; 1:4 and 1:5, plasmid:K2®-transfectant reagent ratios; DNA, pcDNA3.1::flag-actin).



In summary, plasmid:K2®-transfectant reagent efficiently transfected the plasmids tested in L6 cells both in myoblasts and myotubes.