Transient transfection of "DEP-1-HA in pNRTIS21" and "pNRTIS21" (negative control) into NIH3T3 using Lipofectamine (Invitrogen) and Metafectene Pro (Biontex)

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Introduction:

Transient transfection of NIH3T3-cells with "hDEP-1-HA in pNRTIS 21" and "pNRTIS 21" (as negative control) should be done using Lipofectamine (Invitrogen) or Metafectene Pro (Biontex) for comparison. DEP-1-HA-expression and thereby transfection efficiency should then be investigated using immuno-fluorescence (IF). Dep-1-HA will be detected by mouse-anti-HA-antibody and staining with a donkey-anti-mouse-Cy3-labelled secondary antibody.

Materials and methods:

Transfection reagent: Lipofectamine (Invitrogen), Metafectene Pro (Biontex)

Cell-culture-medium: DMEM/F12 and DMEM (Sigma)

8-well-chamber slides (Lab-Tek II Chamber Slides)

Goat serum (Gibco)

Antibodies for IF: anti-HA-antibody (mouse-monoclonal, Nordic Biosite), donkey-anti-mouse-Cy3-labelled (Jackson Immunoresearch)

Experimental procedures / transfection protocol:

Lipofectamine:

-24 h prior to transfection 180,000 NIH3T3-cells were seeded per well of a 8-well chamber slide.

Day of transfection:

-Sol A: 0.15 µg DNA (DEP-1-HA in pNRTIS 21 and pNRTIS 21) were mixed with 30 µl DMEM/F12

-Sol B: 1 µl Lipofectamine (Lipofectamine: Invitrogen) was mixed with 30 µl DMEM/F12

-Sol A and Sol B were mixed and incubated at room temperature for 30 min (complex formation)

-The cells were washed with 300 µl/well DMEM/F12 and then 130 µl/well DMEM/F12 were added

-100 µl DMEM/F12 were added to the complexes and this solution was dropwise added to the cells

-The cells were incubated at 37oC, 5%CO2 over night.

-The next morning the medium was changed to DMEM/F12 + 10% FCS + 50 U/ml penicillin/ 50µg/ml streptomycin and the cells were incubated for further 24 h to allow expression of the protein

Metafectene pro:

-16 h prior to transfection 320,000 NIH3T3-cells were seeded per well of a 8-well chamber slide.

Day of transfection:

-Sol A: 0.14 µg DNA (DEP-1-HA in pNRTIS 21 and pNRTIS 21) were mixed with 13.5 µl DMEM/F12 (mix by pipetting once)

-Sol B: 1.4 µl Metafectene Pro (Metafectene Pro: Biontex; Lot# AD2) was mixed with 13.5 µl DMEM/F12 (mix by pipetting once)

-Sol A was added to Sol B and incubated at room temperature for 20 min (complex formation)

-The medium of the cells was changed to 300 µl DMEM+10% FCS

-After 20 min the complexes were added dropwise to the cells.

-The cells were incubated at 37oC, 5%CO2 for 48 h.

Fixation:

The slides were put on ice for 10 min, washed once with icecold 1x PBS and the fixed with 0.5% PFA in PBS on ice for 30 min.
The slides were washed with 1x PBS (icecold) and permeabilized using 70% EtOH (icecold) on ice for 30 min.

The slides were dried and stored at -20°C until use.

Immunofluorescene: The slides were blocked in 20% Goat serum, 0.05% Tween 20, 1x PBS at 37oC for 2 h. Primary mouse-anti-HA-antibody (100 ng/well) was applied in the same buffer and incubated at 4°C over night. Staining with secondary donkey-anti-mouse-Cy3-antibody was done at room temperature for 1 h. Costaining of the nuclei was done using DAPI in the mounting medium. Pictures were taken with the 20x objective of an epifluorescence microscope.

Results and discussion:

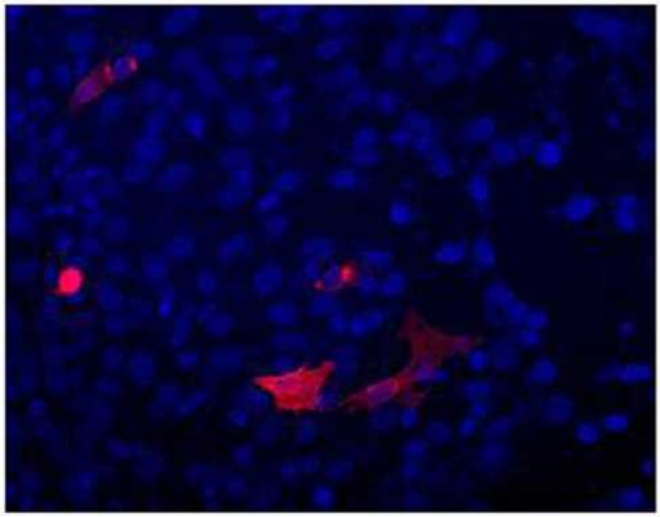
See attached pictures. Transfection efficiency with lipofectamine was poor, the cell of which the picture was taken was among some ~5-10 cells/well which had been transfected. Transfection with Metafectene Pro was much better, here the picture is actually a representative picture for each field. In other words the transfection efficiency was much higher with Metafectene pro (~5%) compared to lipofectamine (~0.1%). The values are approximate.

Conclusion / summary:

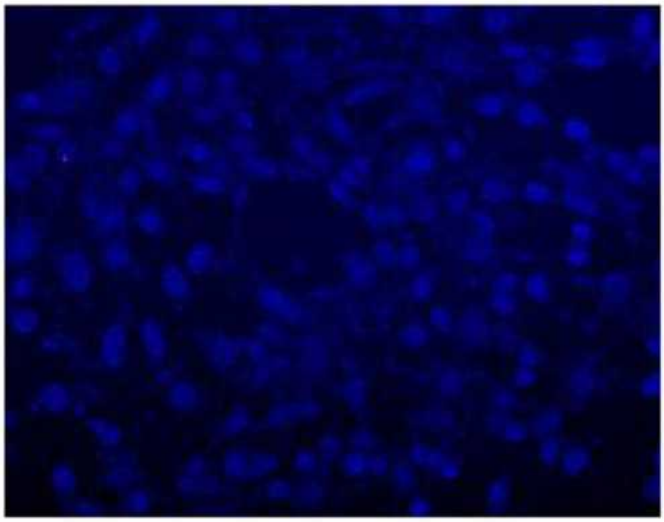
For subsequent transfection of NIH3T3-cells Metafectene Pro will be used instead of Lipofectamine.

Appendix: Tables and/or figures:

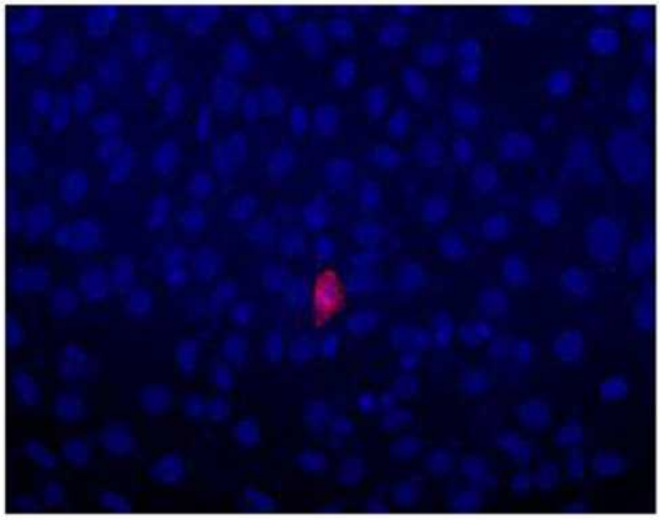
METAFECTENE transfection with protein of interest (DEP-1-HA)



METAFECTENE transfection with empty vector (negative control)



LIPOFECTAMINE transfection with protein of interest (DEP-1-HA)



LIPOFECTAMINE transfection with empty vector (negative control)