Metafectene Method

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Materials:
siRNA to inhibit Caspase-2 expression (Dharmacon)
sterile 6-well tissue culture plates
75 ml cell culture flasks
sterile 5ml polystyrene tubes
trypsin solution
RPMI + 10 % fetal bovine serum
Opti-MEN (Invitrogen)
LNCaP cells (human prostate carcinoma)
Western blotting apparatus
PVDF membrane (pore size 0.2 µm; BIO-RAD)
anti-caspase-2 polyclonal antibody (sc-625, rabbit polyclonal IgG, Santa Cruz Biotechnology)
Western Lightning™ Chemiluminescence Reagent *Plus* (PerkinElmer)

Transfection reagents under examination:
METAFECTENE (Biontex)
Lipid Tr
Lipid O

Confirmation of transfection efficiency for LNCaP cells:
LNCaP cells were grown in a 75 ml cell culture flask in RPMI medium containing 10 % fetal bovine serum to near confluency, then trypsinated and seeded in sterile cell culture 6-well plates (1.8×10^5 LNCaP cells per well in 2 ml of fresh medium). By the time of transfection (typically during the following day), the cells were covering about 40-50% of the plate surface.

Determination of the transfection efficiencies using the different transfection reagents were carried out as follows (all reagents were kept at room temperature):
The solutions below were prepared using 5ml polystyrene tubes:
Solution A: 10 µl of 20 µM siRNA and 170 µl Opti-MEM and
Solution B: 10 µl of transfection reagents and 40 µl Opti-MEM.
Each solution was gently mixed several times by careful pipetting.

The two solutions were combined by pipetting and were then incubated at room temperature for 20-25 min until lipid-siRNA complexes were formed. 70 µl Opti-MEM was then added to each tube and mixed gently by pipetting several times. Next, the solutions were transferred to the wells and mixed gently. The cells were incubated at 37°C in a CO_2 incubator for 48hrs and starved using medium that included 0.5% serum overnight.

The cells were then washed with cold PBS and lysed in Caspase cell lysis buffer, which included 10 mM Tris-HCl, 10 mM NaH_2PO_4, 130 mM NaCl, 1% TritonX-100 and 10 mM NaPPi. Lysates were boiled in SDS sample buffer for 5 min and electrophoresed using 12.5% SDS-polyacrylamide gels (15 µg per lane). Next, the gels were transferred onto a PVDF membrane. After the transfer, the PVDF membrane was blocked with 5% skimmed milk in Tris-buffered saline (TBS) and probed with 1 µg/ml rabbit anti- caspase-2 polyclonal antibody overnight at 4°C. The membrane was incubated in secondary antibody buffer containing HRP-conjugated anti-rabbit IgG antibody. The blot was visualized with Western Lightning™ Chemiluminescence Reagent *Plus*, according to the manufacturer's instructions.

Figure 1. Knock-down of Caspase-2 by siRNA in LNCaP cells using the METAFECTENE reagent.

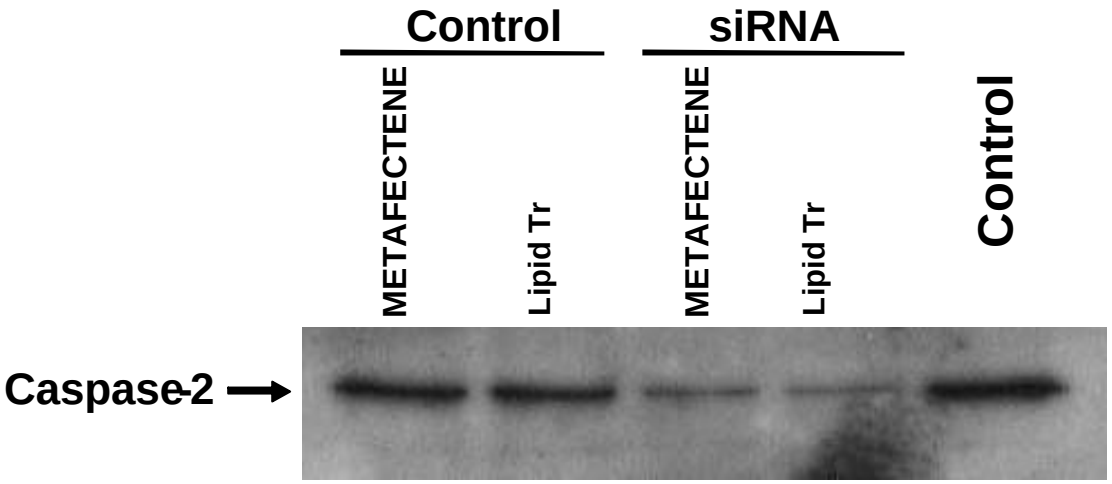

Figure Legend:
LNCaP cells were transfected with siRNA using METAFECTENE or Lipid Tr reagent, according to the conditions described above. Expression of caspase-2 was determined by Western blotting. Equal amounts of cell extract (15 µg protein) with Caspase cell lysis buffer were loaded into each lane. A 48 kDa band of caspase-2 protein clearly decreased after the transfection of siRNA using METAFECTENE or Lipid Tr reagent.

Conclusions:
The expression of caspase-2 clearly decreased after transfection of siRNA using the METAFECTENE reagent was performed (Fig. 1). Lipid Tr was equally effective. These two reagents showed no considerable toxic effects on LNCaP cells, while Lipid O was highly toxic and therefore detrimental to our experiments (data not shown). In summary, the METAFECTENE reagent was effective for the transfection of siRNA.