**METAFECTENE Technical Note**

**Transfection of human primary cultured umbilical vein endothelial cells (HUVEC) with antisense oligodeoxynucleotides (ODN):**

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HUVEC were isolated from freshly collected umbilical cords by treatment with 1.6 U/ml dispase for 30 min at 37°C, seeded into gelatin-coated 6-well plates (2 mg/ml gelatin in 0.1 mM HCl for 30 min at ambient temperature) and cultured in medium M199 containing 20% fetal bovine serum, 50 U/ml penicillin, 50 µg/ml streptomycin, 10 U/ml nystatin, 5 mM HEPES and 5 mM TES, 1 µg/ml heparin, and 40 µg/ml endothelial cell growth factor. For transfection cells were grown to 90-95% confluence (normally within 4 to 5 days) referring to the covered growing area (estimated optically by light microscopy).

Transfection with METAFECTENE was performed as follows (all reagents should be brought to ambient temperature): 10 µl METAFECTENE was mixed with 100 µl of medium M199 free of serum and antibiotics in a 1,5 ml Eppendorf cup (solution A). 5 µg antisense-ODN and 100 µl medium M199 free of serum and antibiotics were mixed in an additional cup (solution B). Subsequently, both solutions were combined and mixed gently by carefully pipetting several times up and down and incubated at ambient temperature for 20 min to form the lipid-DNA complexes.
During the incubation period the conditioned medium was replaced with 2 ml of fresh Optimem 1 medium (Gibco/Invitrogen Corporation) per well. Then the lipid-DNA complexes were added to the cells and mixed gently by carefully pipetting several times up and down. The transfection mixture was removed after 3.5 h of incubation at 37°C in an incubator gassed with 5% $CO_2$, replaced by fresh medium M199 followed by a further 24 h incubation of the cells in the incubator.

Thereafter cells were homogenized by five cycles of freeze-thawing in liquid nitrogen and at 37°C (heater). Protein extracts (20 µg protein per lane) were separated by denaturing 10% polyacrylamid gel eletrophoresis in the presence of SDS according to standard protocols and then transferred to a polyvinylidene fluoride transfer membrane. Visualization of the glutathione peroxidase (GSH-Px) protein bands was achieved by using the chemiluminescent method followed by exposure to an autoradiography film.

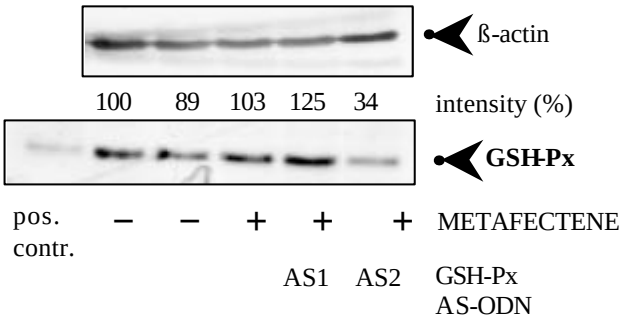


**Figure**: Effect of a specific antisense-ODN (AS2) against glutathione peroxidase (GSH-Px) in human endothelial cells after transfetion with the METAFECTENE transfection reagent. The unspecific antisense ODN AS1 served as a control. Typical Western blot analysis with the relative intensities (%), as judged by densitometry, as compared to the basal GSH-Px protein content in the cultured endothelial cells. Qualitatively identical results were obtained with at least two further batches of HUVEC. Loading and transfer of equal amounts of protein in each lane was verified by reprobing the membrane with an anti-ß-actin antibody.