An Ultrasensitive Biosensor for Detection of Femtogram Levels of the Cancer Antigen AGR2 Using Monoclonal Antibody Modified Screen-Printed Gold Electrodes

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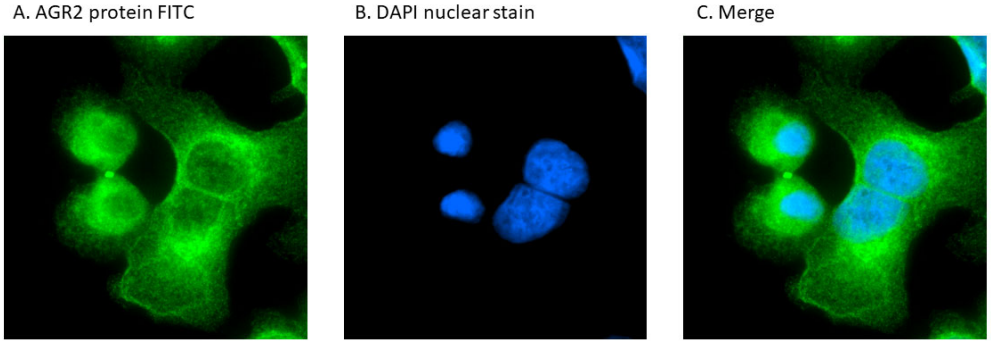

Figure S1. Immunohistochemical expression of AGR2 protein in A549 cells. The A549 cells were fixed with 4% (v/v) paraformaldehyde in PBS at RT for 15 min, washed with PBS three times, and permeabilized using 0.25% triton X-100 in PBS at RT for 10 min. Then, the cells were again washed with PBS three times and blocked with 3% BSA in PBS for 1 h. The primary antibody was incubated at appropriate dilution (typically 1:1000) overnight at 4°C. Alexa Fluor 488 goat anti-mouse (Invitrogen, USA) secondary antibody was incubated at RT for 1 h. Coverslips were washed three times with PBS in between each step. Cells were incubated in DAPI (image B and C) (Invitrogen, USA) diluted at 1:10,000 with dH_2O for 5 min to stain the nucleus. An additional 3 washes with dH_2O for 5 min were performed. A single drop of Fluorescence Mounting Medium (S3023, Dako, Denmark) was used to mount the cells on the slide. The fluorescent signal (image A and C) was detected using a Zeiss Axioplan 2 microscope (63x or 100x oil immersion objective). Images were acquired by Micro-Manager 1.4 software. Images were processed in ImageJ 2.0 software.