

En face aorta immunofluorescent staining

For en face immunostaining, the mice are perfused with 4% PFA. The whole aorta is excised, gently cleaned of perivascular fat and fixed for 15 minutes with 4% PFA. Next, the aorta is incubated for 3 h in blocking (5% goat serum, 1% BSA in PBS) or blocking permeabilising buffer (0.3% Triton X-100, 5% goat serum, 1% BSA in PBS) and then incubated overnight with the antibodies diluted in 3% BSA (for Nrf2 Ab 1:100). After that, the aorta is washed 3 times and, if needed, incubated with the host-specific antibodies conjugated with Alexa Fluor (AF) fluorophores (dilution 1:1000; IgG H+L, Life Technologies) and DAPI (1 µg/ml, Sigma-Aldrich) for 2 h at RT. After 3 series of washing, the aorta is opened, intima facing up and mounted with Dako Fluorescence Mounting Medium (Dako). High-resolution images are taken using a meta laser scanning confocal microscope (LSM-880; Carl Zeiss).

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