

Microwave Irradiation of Cryostat Sections Accelerates and Improves Nitric Oxide Synthase Staining,

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A recent increase in nitric oxide synthase research demands a sensitive and reliable morphological detection of its enzyme, nitric acid synthase (NOS). As an antibody against NOS is not readily available, a widely used histochemical stain for NADPH specifically depicts the distribution of NOS in paraformaldehyde-fixed frozen sections. We found that irradiating the crysostat section in a microwave oven significantly improved the detection sensitivity of this method. Various power settings and durations of microwave irradiation were tested with a domestic oven utilizing cryostat sections of paraformaldehyde fixed rat brain and heart as models. NADPH specifically stained cytoplasm of neurons, nerve terminals, endothelial cells and vascular smooth muscle cells. Microwave treatment at high power setting (600 W) significantly increased the staining abundance and density while lowering the background. In particular, many more small diameter nerve fibers were visualized in comparison with the conventional procedure. Microwave irradiation shortened the drying period of the cryostat sections from 30 min to 1 hr to about 10 min and enhanced the tissue section attachment. Cell morphology appeared to improve. The beneficial effects of microwave irradiation might be due to the unmasking of NADPH molecules, activation of enzyme activities, minimizing the loss of activity by shortening the drying time or a combination of the above. We conclude that microwave irradiation of cryosections significantly improves the efficiency of NOS staining.