Tumor development and pimonidazole immunohistochemistry

MDA-MB-231 breast cancer cells were implanted into the mammary fat pad of Nu/Nu mice and experiments were carried out at the end of tumor development (1 cm^3). Animals were injected with pimonidazole hydrochloride (60 mg/ml) (HPI, Inc., Burlington, MA) I.P. two hours prior to tumor extraction to identify hypoxic tissue. Extracted tumors were formalin fixed and paraffin embedded for immunohistochemical processing. Following standard deparaffinization protocols, pimonidazole was detected with rabbit primary anti-sera (Hypoxyprobe, PAb2627) and a universal secondary antibody. Slides were counterstained with Hematoxylin. The slides were scanned in the Moffitt Analytical Microscopy Core Facility (AMC) using an Aperio ScanScope XT digital slide scanner (Aperio).