**Immunohistochemistry Protocol Notes:**

*Note: Do not use tissues fixed overnight in PFA, the antibody will not work.*

The best fixative is MEMFA. 10X stock solution for MEMFA: 1M MOPS, 20mM EGTA, 10mM MgSO4, 38% Formaldehyde.

Fixation 1 hour, 2 x 15 min methanol. Following this protocol, tissues may be stored in methanol at -20°C indefinitely or immediately embedded in paraplast. Best results on paraffin sections 6-10 micron thick.

Staining following deparaffinization in xylene and a row of alcohol wash two times in water. Block in 2% Boehringer Mannheim reagent in 0.1M maleic acid, pH 7.5, 150mM sodium chloride for one hour at RT.

Dilute NB110-12933 in same blocking reagent and incubate overnight at 4°C or for 5 hours. Wash three times in PBS, 10 min each.

Incubate with conjugated secondary antibody. Develop with appropriate reagent (if applicable).

For sections always use Digene silanated slides or Superfrost plus from Fisher as sometimes you may need to boil sections in 6M urea for 5-6 min in microwave at 80% power following deparaffinization to increase signal. That is especially useful if tissues were fixed in PFA.

Sometimes it is necessary to predeplete antibody on hyperfixed tissues to lower background (especially for staining species other than frog and for whole-mounts).