Recommended Immunohistochemistry Protocol:

The following protocol is a recommendation only, and MBL makes no guarantee of the results:

Tissue Preparation:	Formalin fixation and embedding in paraffin wax
Tissue Sectioning:	Make 4-µm sections and place on pre-cleaned and charged microscope slides. Heat in a tissue-drying oven for 45 minutes at 60°C.
Deparaffinization:	Wash dry slides in 3 changes of xylene – 5 minutes each @ RT
Rehydration:	Wash slides in 3 changes of 100% alcohol – 3 minutes each @ RT Wash slides in 2 changes of 95% alcohol – 3 minutes each @ RT Wash slides in 1 change of 80% alcohol – 3 minutes @ RT Rinse slides in gentle running distilled water – 5 minutes @ RT
Antigen retrieval:	Steam slides in 0.01 M sodium citrate buffer, pH 6.0 at 99-100°C - 20 minutes Remove from heat and let stand at room temperature in buffer - 20 minutes Rinse in 1X TBS with Tween (TBST) – 1 minute @ RT
Immunostaining:	(Do not allow tissues to dry at any time during the staining procedure) Apply a universal protein block – 20 minutes @ RT Drain protein block from slides, apply diluted primary antibody – 45 minutes @ RT Rinse slides in 1X TBST - 1 minute @ RT Apply a biotinylated anti-rabbit IgG (H+L) secondary – 30 minutes @ RT Rinse slides in 1X TBST - 1 minute @ RT Apply alkaline phosphatase streptavidin – 30 minutes @ RT Rinse slides in 1X TBST - 1 minute @ RT Apply alkaline phosphatase chromogen substrate – 30 minutes @ RT Wash slides in distilled water – 1 minute @ RT
Dehydrate:	(This method should only be used if the chromogen substrate is alcohol insoluble (e.g. Vector Red, DAB) Wash slides in 2 changes of 80% alcohol – 1 minute each @ RT Wash slides in 2 changes of 95% alcohol – 1 minute each @ RT Wash slides in 3 changes of 100% alcohol – 1 minute each @ RT Wash slides in 3 changes of xylene – 1 minute each @ RT Apply coverslip

- The sodium citrate buffer, TBST, universal block and antibody diluent are purchased from Dako, and the secondary antibodies and chromogen (usually Vector Red) are obtained from Vector.

- Our tissues are fixed in 10% neutral buffered formalin. The ratio of buffered formalin to tissue volume is 10:1 for approximately 18 hours. Formalin has a very slow diffusion coeffient so the tissue needs to be no more than about the thickness of a nickel.