



<b>Catalog Number:</b>	RA30025	<b>Host:</b>	Rabbit
<b>Product Type:</b>	Affinity Purified Antibody	<b>Species Reactivity:</b>	Human
<b>Immunogen Sequence:</b>	KLH Coupled Synthetic Peptide-internal domain of human Wnt5b	<b>Format:</b>	PBS, PH 7.7 containing 0.01% sodium azide. Concentration: 1 mg/ml.
<b>Applications:</b>	Immunohistochemistry: 2.5 µg/ml  Dilutions listed only as a recommendation. Optimal dilution should be determined by researcher.		
<b>References:</b>	Long term: -70°C; Short term: +4°C. . Repeated freeze/thaw cycles compromise the integrity of the antibody.		

### Application Notes

This antibody is designed for primarily for IHC staining of Paraffin Embedded Tissue.

#### Immunohistochemistry Protocol

*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 at Room Temperature.

*Rehydration:* Wash slides in 3 changes of 100% alcohol – 3 minutes each at Room Temperature. Wash slides in 2 changes of 95% alcohol – 3 minutes each at Room Temperature Wash slides in 1 change of 80% alcohol – 3 minutes at Room Temperature. Rinse slides in gentle running distilled water – 5 minutes at Room Temperature.

*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 at Room Temperature.

*Immunostaining:* (Do not allow tissues to dry at any time during the staining procedure). Apply a universal protein block – 20 minutes at Room Temperature. Drain protein block from slides, apply diluted primary antibody – 45 minutes at Room Temperature. Rinse slides in 1X TBST - 1 minute at Room Temperature. Apply a biotinylated anti-rabbit IgG (H+L) secondary – 30 minutes at Room Temperature. Rinse slides in 1X TBST - 1 minute at Room Temperature. Apply alkaline phosphatase streptavidin – 30 minutes at Room Temperature. Rinse slides in 1X TBST - 1 minute at Room Temperature. Apply alkaline phosphatase chromogen substrate – 30 minutes at Room Temperature. Wash slides in distilled water – 1 minute at Room Temperature

*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 at Room Temperature. Wash slides in 2 changes of 95% alcohol – 1 minute each at Room Temperature. Wash slides in 3 changes of 100% alcohol – 1 minute each at Room Temperature. Wash slides in 3 changes of xylene – 1 minute each at Room Temperature. Apply coverslip

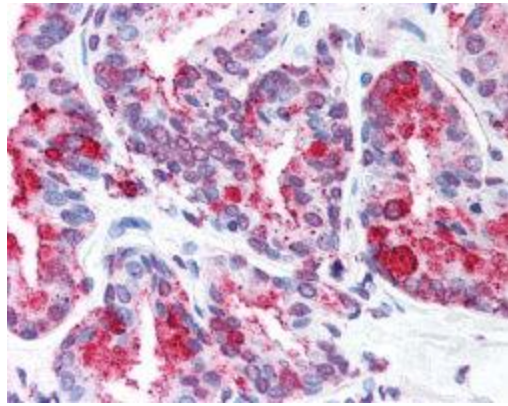
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*Image: Wnt5b staining of Human Prostrate. Formalin-Fixed Paraffin-Embedded (FFPE).*



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