



Catalog Number:	RA30062	Host:	Rabbit
Product Type:	Affinity Purified Antibody	Species Reactivity:	Human
Immunogen Sequence:	KLH-conjugated synthetic peptide corresponding to amino acids at the C-terminus of human CARD8.	Format:	50 ul. PBS, 0.02% sodium azide. Concentration: 1.0 mg/ml.
Applications:	Immunohistochemistry: 5.0 µg/ml (Paraffin Embedded Only). Western blot: 1.0 ug/ml.		
	<i>Dilutions listed only as a recommendation. Optimal dilution should be determined by researcher.</i>		
Storage:	Store at: +4°C. Do not freeze.		

Application Notes

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

*[Goat anti-rabbit IgG. Biotin-Catalog#:GT30001](#)

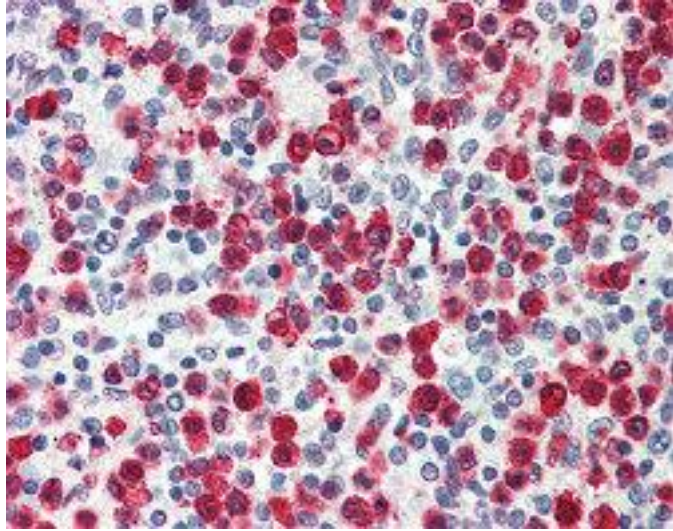
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Image: Human Spleen (formalin-fixed, paraffin-embedded) stained with CARD8 antibody at 5.0 ug/ml followed by [Goat anti-rabbit IgG, Biotin-Catalog#:GT30001](#), alkaline phosphatase-streptavidin and chromogen.



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