



Catalog Number:	RA30023	Host:	Rabbit
Product Type:	Affinity Purified Antibody	Species Reactivity:	Human, Rat, Zebrafish
Immunogen Sequence:	Synthetic peptide containing amino acids 204-215 of Human PACRG protein.	Format:	Liquid. 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2 with 0.01% (w/v) Sodium Azide. Concentration of 1 mg/ml.
Applications:	Immunohistochemistry: 1.5-2.5 µg/ml Immunofluorescence-0.3-2.0 µg/ml Western Blot-0.3-2.0 µg/ml ELISA-0.1-0.5 µg/ml		
	Dilutions listed only as a recommendation. Optimal dilution should be determined by researcher.		
References:	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 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

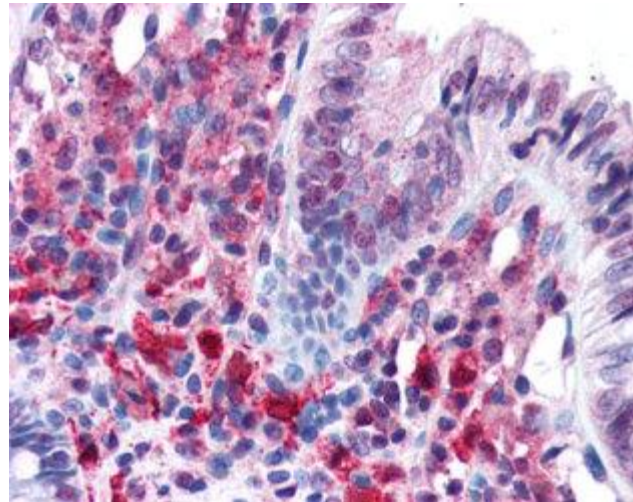
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Image: PACRG Staining of human colon: Formalin-Fixed Paraffin-Embedded (FFPE).



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