



Catalog Number:	RA30033	Host:	Rabbit
Product Type:	Affinity Purified Antibody	Species Reactivity:	Human, Bovine, Chicken, Frog, Mouse, Pig, Rat, Zebrafish
Immunogen Sequence:	Synthetic peptide-Amino acids 417-425 of human SMAD3 protein. Immunogen modification: pSer423/425.	Format:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2 with 0.01% (w/v) Sodium Azide. Concentration 1 mg/ml.
Applications:	Immunohistochemistry: 2.5 µg/ml Western Blot: 1:2000 - 1:20000 ELISA: 1:25000 - 1:100000		
References:	Dilutions listed only as a recommendation. Optimal dilution should be determined by 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. It can also be used for Western Blot and ELISA

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

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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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