



Catalog Number: RA30039	Host: Rabbit
Product Type: Affinity Purified Antibody	Species Reactivity: Mouse, Human, Rat
Immunogen Sequence: Synthetic peptide from the C-terminus of mouse MAPK1.	Format: PBS, pH 7.4, 0.05% sodium azide. Concentration 1 mg/ml.
Applications: Immunohistochemistry: 5 µg/ml (Paraffin Embedded Only). Western Blot*	
*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

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: ERK2 staining of Heart Tissue. Paraffin-Embedded Formalin-Fixed Tissue.

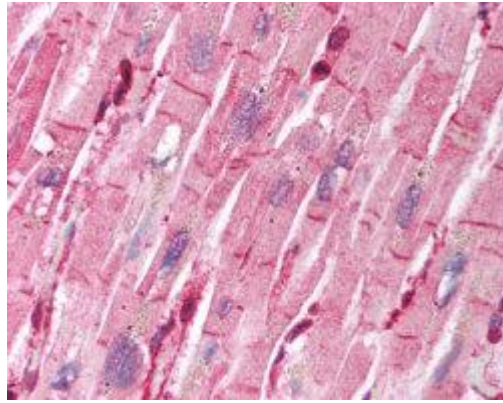
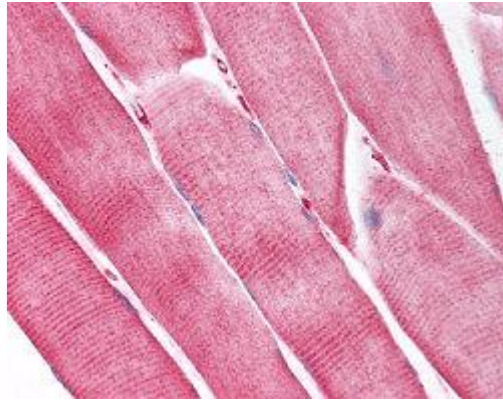


Image: ERK2 staining of Skeletal Muscle Tissue. Paraffin-Embedded Formalin-Fixed Tissue.



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