



Catalog Number:	GT41030	Host:	Goat
Product Type:	Affinity Purified Antibody	Species Reactivity:	Human, Mouse, Rat
Immunogen Sequence:	Peptide with sequence C-TKESESTNSVRLML, from the C Terminus of the protein sequence according to NP_060142.3.	Format:	Liquid 200 ul. (0.5 mg/ml). Tris saline, 0.02% sodium azide, pH 7.3, 0.5% BSA
Applications:	Immunohistochemistry: 4.0-6.0 µg/ml (paraffin embedded tissue only). Peptide ELISA: 1:8,000.		
	*Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.		
Storage:	Aliquot and store at -20°C. Avoid repeated freeze-thaw cycles.		

Application Notes

Immunohistochemistry:

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 horse-anti-goat IgG , biotin secondary (HO30002)*– 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

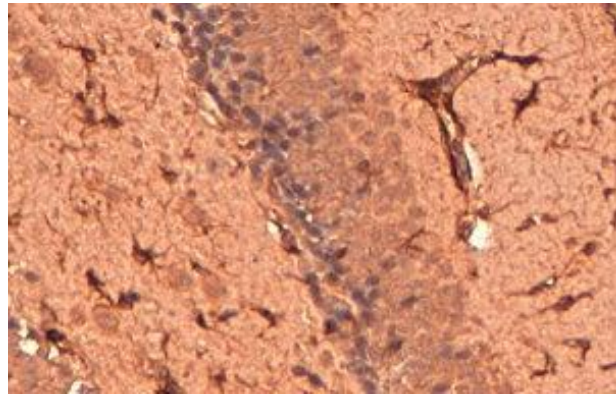
Dehydrate: (This method should only be used if the chromogen substrate is alcohol insoluble (e.g. Vector Red, DAB). Wash slides in 2 changes of 80% alcohol – 1 minute each at Room Temperature. Wash slides in 2 changes of 95% alcohol – 1 minute each at Room Temperature. Wash slides in 3 changes of 100% alcohol – 1 minute each at Room Temperature. Wash slides in 3 changes of xylene – 1 minute each at Room Temperature. Apply coverslip

* [Horse anti-goat IgG. Biotin \(catalog# HO30002\)](#)

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Image: TRPM7 (dilution: 4.0 µg/ml) staining of paraffin embedded Mouse Hippocampus. Steamed antigen retrieval with citrate buffer pH 6, HRP-staining. Similar results were obtained after antigen retrieval at pH 9.



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