



Catalog Number:	RA30000	Host:	Rabbit
Product Type:	Affinity Purified Antibody	Species Reactivity:	Human
Immunogen Sequence:	Synthetic peptide - KLH conjugated 3rd cytoplasmic domain of human 5-HT1E.	Format:	Phosphate buffered saline containing 0.1% sodium azide.
Applications:	Western Blot 1:100 ~1:1,000 (ECL) Immunofluorescence 1:100 ~1:250* Immunohistochemistry: 16 – 24 µg/ml		

Dilutions listed only as a recommendation. Optimal dilution should be determined by investigator. *May see multiple smaller MW bands, as well.

Storage: Store frozen. Aliquot as undiluted antisera and immediately place at -20°C. Antisera may have become trapped in top of vial during shipping. Centrifugation of vial is recommended before opening. Stable for at least 6 months at -20°C. Repeated freeze/thaw cycles compromise the integrity of the antiserum.

References:

1. Gu, Y.Z., and Schonbrunn, A. Coupling specificity between somatostatin receptor sst2A and G proteins: Isolation of the receptor-G protein complex with a receptor antibody. *Molecular Endocrinology*. 11: 527-537, 1997.
2. Reubi, J.C., Kappeler, A., Waser, B., Laissue, J., Hipkin, R.W., Schonbrunn, A. Immunohistochemical localization of somatostatin receptor sst2A in human tumors. *American Journal of Pathology*. 153: 233-245, 1998.

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

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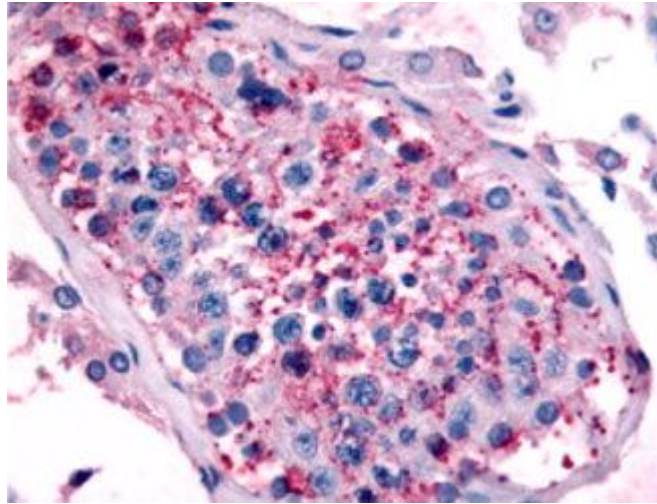
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Dehydrate: (This method should only be used if the chromogen substrate is alcohol insoluble (e.g. Vector Red, DAB). Wash slides in 2 changes of 80% alcohol – 1 minute each at Room Temperature. Wash slides in 2 changes of 95% alcohol – 1 minute each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

Image: 5HT-1E staining of paraffin embedded testis.



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