NEUROMICS

P2YR8 Data Sheet

Catalog Number:	RA30006	**Host:**	Rabbit
Product Type:	Affinity Purified Antibody	**Species Reactivity:**	Human
Immunogen Sequence:	Synthetic peptide - KLH conjugated. C-terminal cytoplasmic domain of human.	**Format:**	Phosphate buffered saline containing 0.1% sodium azide. Concentration of 1 mg/ml.
Applications:	Immunohistochemistry: 20 µg/ml Dilutions listed only as a recommendation. Optimal dilution should be determined by		
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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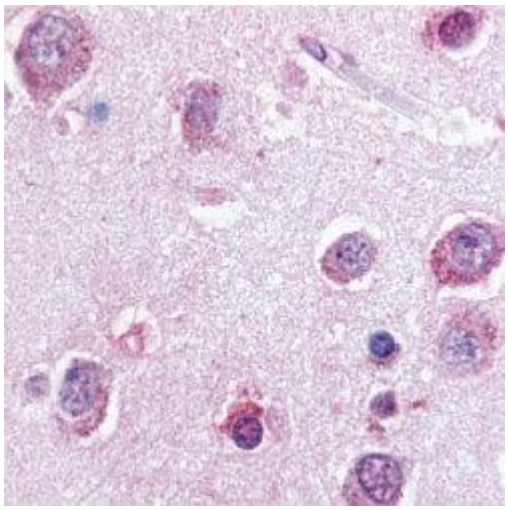
Note on Orphan Receptors:
Since supporting data from binding studies and ligand-mapping studies are not available, and evaluation of the specificity and validity of our IHC findings relies more heavily on cross-concordant findings with multiple antibodies and on gene expression studies such as Northern-blot analysis or in situ hybridization. Generally, proteins that are detectable within a particular type of tissue by Northern-blot analysis are also detectable by IHC. Although it is possible for a gene to be transcribed within a tissue but not translated, our experience has been that a positive finding on a human tissue Northern blot generally correlates with the ability to detect the protein within that tissue by IHC. Northern analysis can, however, underestimate the presence of low-copy mRNAs or those that are expressed by only a small subset of cells within a tissue.

In situ hybridization (ISH) is a more sensitive method for the detection of low-abundance genes, because mRNA can be localized to individual cells within a tissue sample. However, this method does not lend itself readily to high-throughput, first-pass screening in formalin-fixed, paraffin-embedded tissue specimens, and many cell types may produce mRNAs that are below the limit of sensitivity of detection by colorimetric or radiometric ISH

Image: P2YR8 staining of paraffin embedded human brain tissue.



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