NEUROMICS

Neurogranin precursor Data Sheet

Catalog Number:	GT41024	**Host:**	Goat
Product Type:	Affinity Purified Antibody	**Species Reactivity:**	Human, Rat, Mouse
Immunogen Sequence:	Peptide with sequence C-KIKSGERGRKG, from the internal region of the Neurogranin precursor protein sequence according to NP_006167.1.	**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).
Western Blot: 0.1-0.3 µg/ml
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

Western blot:
Approx 15kDa band observed in Human Brain (Cerebral Cortex and Hippocampus), and in Mouse Brain and Rat Brain lysates (calculated MW of 7.6kDa according to NP_006167.1). There is also an additional 16kDa band. The observed molecular weights correspond to earlier findings in literature with different antibodies (Li et al, J Biol Chem. 1999 Jan 15;274(3):1294-300; PMID: 9880498). Recommended concentration: 0.1-0.3µg/ml.

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

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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

* Horse anti-goat IgG, Biotin (catalog# HO30002)

Image: Neurogranin precursor (dilution: 4.0 µg/ml) staining of paraffin embedded Human Cortex. Steamed antigen retrieval with citrate buffer pH 6, AP-staining.

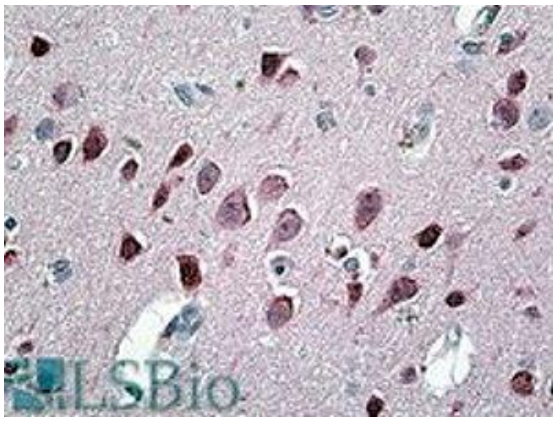
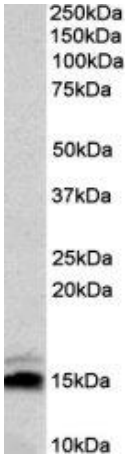


Image: Neurogranin precursor (dilution: 0.1µg/ml) staining of Mouse Brain lysate (35µg protein in RIPA buffer). Primary incubation was 1 hour. Detected by chemiluminescence.



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