

Immunohistochemistry Sample Contract Research Report GPCR Orphan-U

Summary:

Orphan-U is a G-protein coupled receptor with an unknown ligand. This GPCR shows sequence homology to receptors that are involved in DNA replication and repair in response to various types of cell injury. Expression studies have reported positive mRNA in the pituitary, but peripheral tissues and normal brain were negative in Northern blots. Antibody LP-GPCR-U was evaluated in sections of normal hippocampus and hippocampus from patients with Alzheimer's disease.

Patients with Alzheimer's disease showed strong, increased staining within neurons, neurofibrillary tangles, dystrophic neurites, and subsets of senile plaques. Normal hippocampus sections, in contrast, were largely negative for staining. These results suggest that this antibody is a potential marker or target for Alzheimer's disease, and merit further studies on a larger series of patients and neurological diseases.

Methods:

Four affinity-purified anti-peptide rabbit polyclonal antibodies were generated by LifeSpan and tested in immunohistochemistry on formalin-fixed, paraffin archival human peripheral multi-tissue and multi-brain sections at concentrations of 2.5, 5, 10, and 20 ug/ml. Antibody LP-GPCR-U at a concentration of 2.5 ug/ml showed the best signal to background and was used in further studies. The antibody was used as the primary antibody, and the principal detection system consisted of a Vector anti-goat secondary (BA-5000) and a Vector ABC-AP kit (AK-5000) with a Vector Red substrate kit (SK-5100), which produced a fuchsia-colored deposit.

Negative controls consisted of running an IgG-isotype control antibody on serial sections and running all procedures in the absence of primary antibody. Both the IgG-isotype control antibody and no primary control sections were negative for staining across all tissues tested.

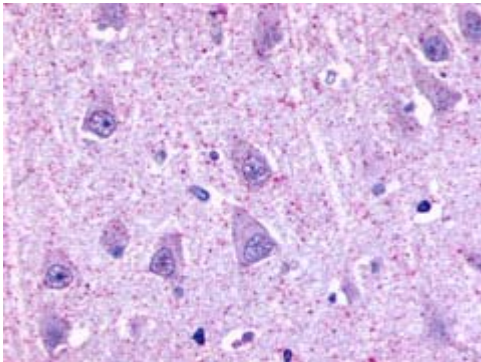
Tissues were also stained with positive control antibodies (CD31 and vimentin) to ensure that the tissue antigens were preserved and accessible for IHC analysis. Only tissues that were positive for CD31 and vimentin staining were selected for the remainder of the study. The negative control consisted of performing the entire immunohistochemical procedure on adjacent sections in the absence of primary antibody. Slides were imaged with a DVC 1310C digital camera coupled to a Nikon microscope. Images were stored as TIFF files with Adobe Photoshop.

Results:

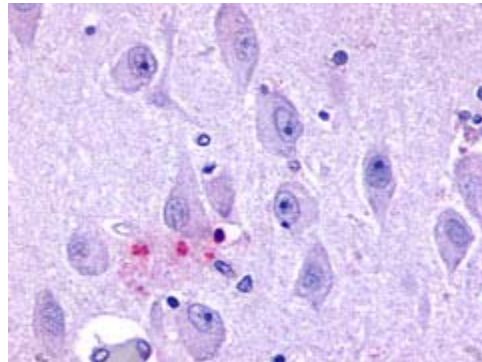
Brain, Normal Hippocampus

Sample 1: This sample of normal hippocampus was obtained from a 46-year-old male who died of complications of cervical spinal cord syringomyelia.

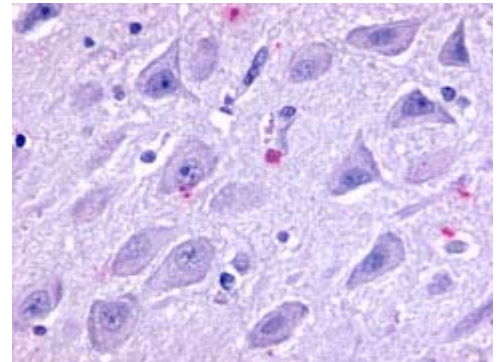
Neurons in areas CA2 through CA4, neurons in the dentate gyrus, neurons of the subiculum, and neurons of the adjacent entorhinal cortex were negative. Neuropil and neurons in area CA-1 showed blush to faint staining. Astrocytes and oligodendrocytes were negative. Adjacent choroid plexus epithelium and adjacent ependymal cells were negative.



001: Neurons within Area CA1 40X

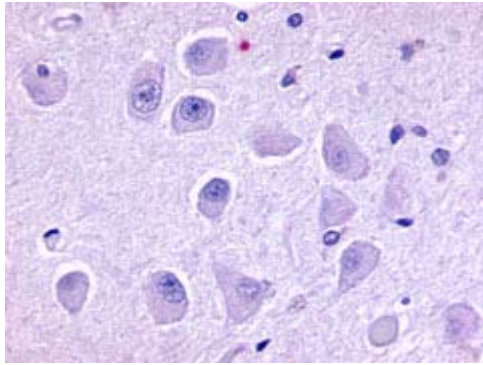


002: Neurons within Area CA2 40X

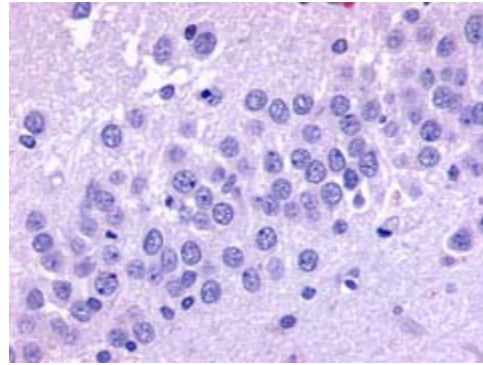


003: Neurons within Area CA3 40X

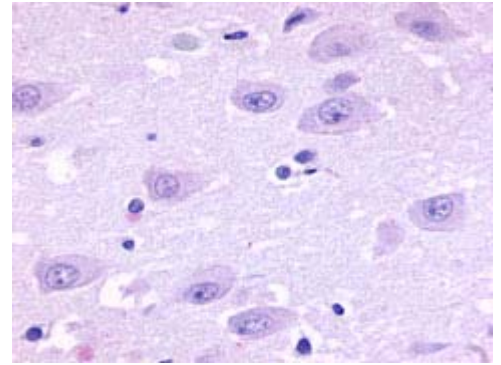
Immunohistochemistry Sample Contract Research Report GPCR Orphan-U



004: Neurons within Area CA4 40X



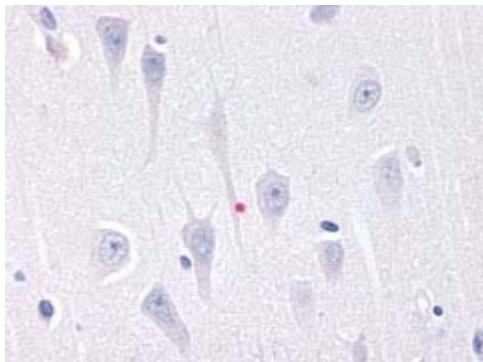
005: Granule Neurons within Dentate Gyrus 40X



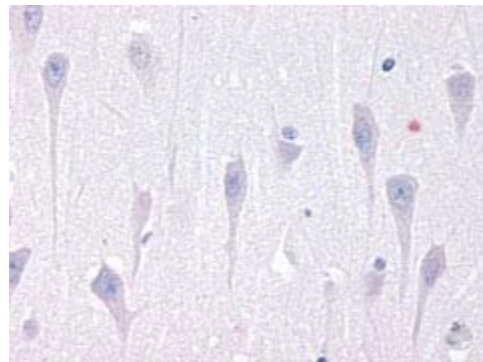
006: Neurons within Subiculum 40X

Sample 2: This sample of normal hippocampus was obtained at autopsy from a 50-year-old female who died of bronchopneumonia.

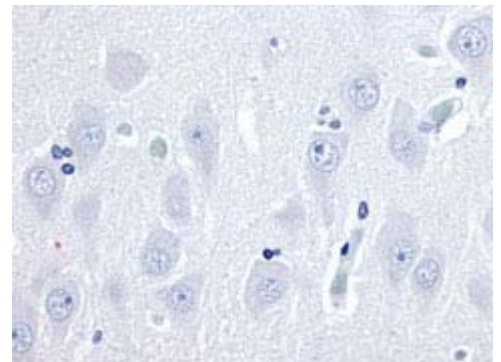
Neurons in areas CA1 through CA4, the dentate gyrus, the subiculum, and within the adjacent entorhinal cortex were negative. Astrocytes and oligodendrocytes were negative. Adjacent choroid plexus epithelium and adjacent ependymal cells were negative.



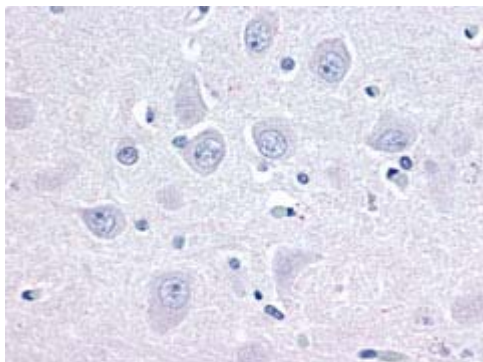
007: Neurons within Area CA1 40X



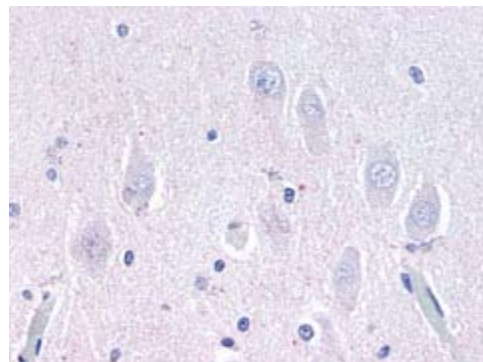
008: Neurons within Area CA1 40X



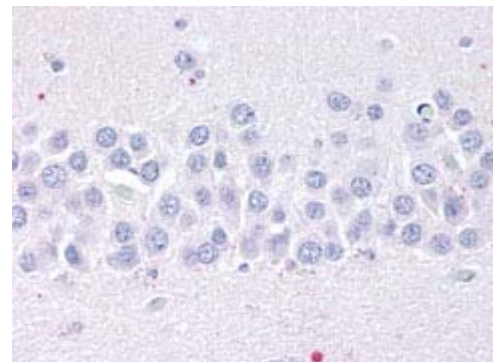
009: Neurons within Area CA2 40X



010: Neurons within Area CA3 40X



011: Neurons within Area CA4 40X



012: Granule Neurons within Dentate Gyrus 40X

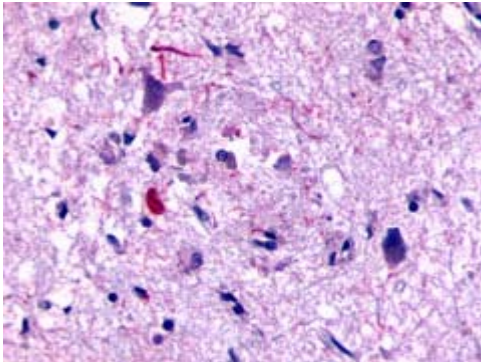
Brain, Alzheimer's Disease

Sample 1: This sample of brain was obtained from a 77-year-old female who died of acute staphylococcal pneumonia.

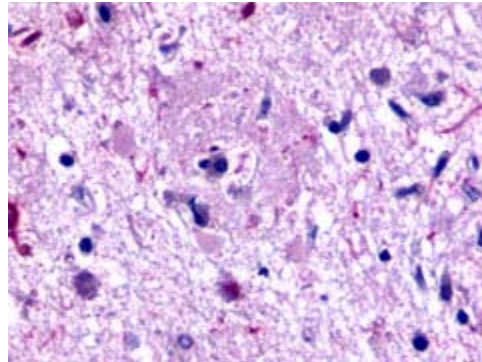
Neurons showed strong staining, particularly those containing neurofibrillary tangles within area CA1, the entorhinal cortex, and subiculum. Occasional senile plaques showed strongly stained dystrophic neurites. Amyloid cores were negative. Neuropil threads were strongly positive in the entorhinal cortex and isocortex. Astrocytes showed blush staining, and oligodendrocytes

Immunohistochemistry Sample Contract Research Report GPCR Orphan-U

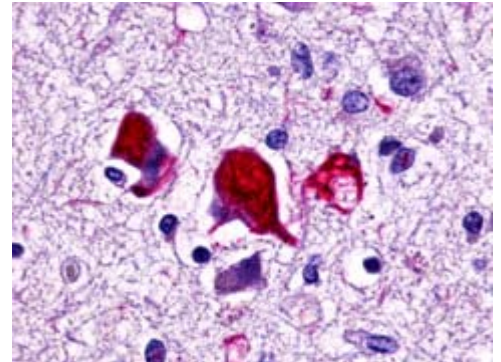
and microglia were negative. Endothelial cells within vessels, pericytes, and vascular smooth muscle were negative. Compared to samples of normal cortex, this sample of Alzheimer's disease showed strong positivity of neurofibrillary tangles, neuropil threads, and dystrophic neurites within senile plaques.



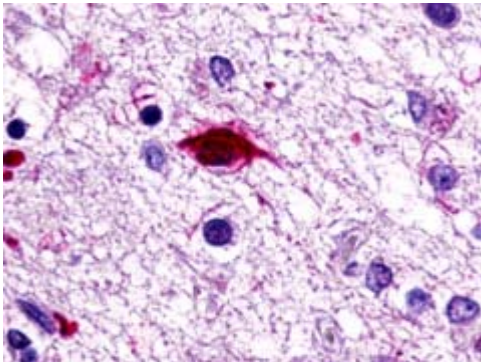
013: Senile Plaque 40X



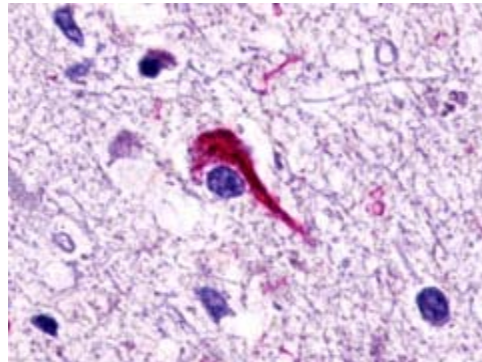
014: Senile Plaque 40X



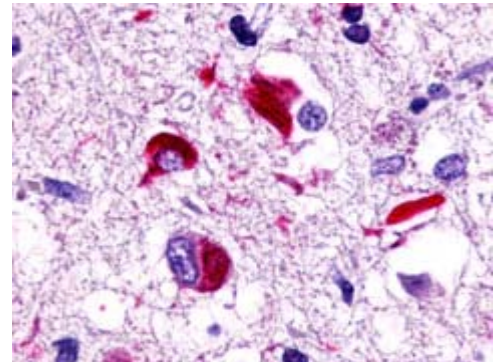
015: Neurofibrillary Tangles 60X



016: Neurofibrillary Tangles 60X



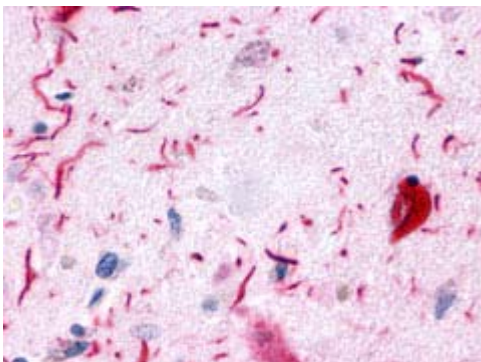
017: Neurofibrillary Tangles 60X



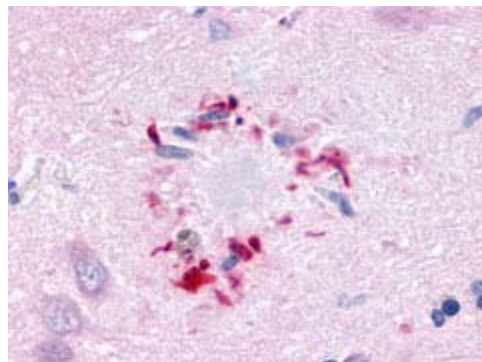
018: Neurofibrillary Tangles 60X

Sample 2: This sample of brain was obtained at autopsy from an 83-year-old male.

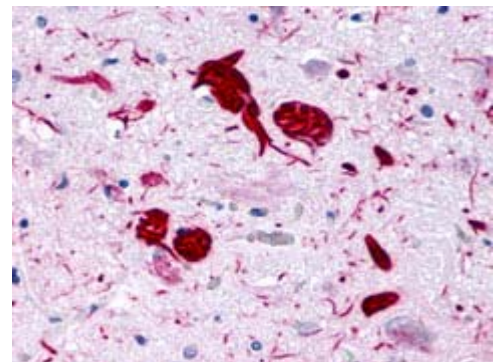
Neurons showed strong staining, particularly those containing neurofibrillary tangles within area CA1-4, the entorhinal cortex, and subiculum. Senile plaques frequently showed strongly stained dystrophic neurites. Amyloid cores were negative. Neuropil threads were strongly positive in the entorhinal cortex and isocortex. Astrocytes showed blush staining, and oligodendrocytes and microglia were negative. Endothelial cells within vessels, pericytes, and vascular smooth muscle were negative. Compared to samples of normal cortex, this sample of Alzheimer's disease showed strong positivity of neurofibrillary tangles, neuropil threads, and dystrophic neurites within senile plaques.



019: Senile Plaque, Neurofibrillary Tangle and Neuropil Threads 60X

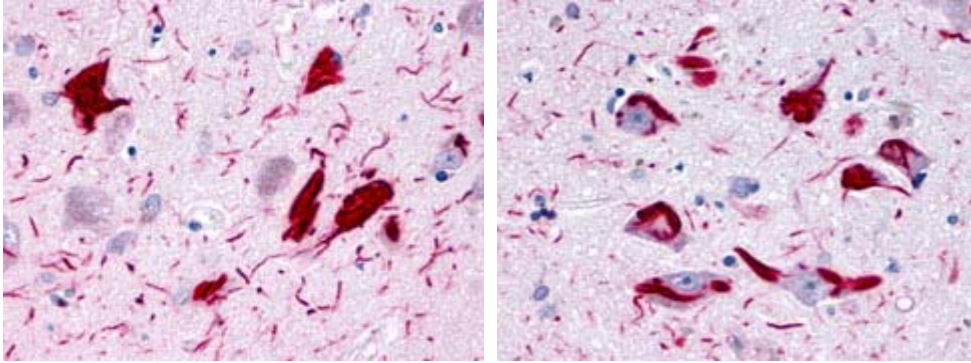


020: Senile Plaque and Dystrophic Neurites 60X



021: Neurofibrillary Tangle, Dystrophic Neurites, and Neuropil Threads 40X

Immunohistochemistry Sample Contract Research Report GPCR Orphan-U



022: Neurofibrillary Tangle and Neuropil Threads 40X 023: Neurofibrillary Tangle and Neuropil Threads 40X

Note: Although these results have been reviewed by a Pathologist, these studies are to be used for research purposes only and are not intended for clinical patient care. These results were obtained on a limited series of samples and tissues and therefore cannot be construed to represent a comprehensive picture of localization across the body. Further studies are recommended if one wishes to determine the true prevalence of staining within a particular tissue or disease with this antibody, or to obtain a more comprehensive distribution of staining across a broader variety of tissues.