

Immunohistochemistry Sample Report for Antibody LP-GPCRA on Colon and Breast Carcinomas and Normal Samples

Summary

Antibody LP-GPRA, a Western positive rabbit polyclonal antibody targeting the amino terminus of the GPCR Orphan-A protein, was evaluated in immunohistochemistry on normal colon and breast samples and three of each of adenocarcinomas of the colon and breast. Compared to normal samples, three colon carcinomas showed strong, increased staining within greater than 90% of the tumor cells. In the breast carcinomas, two of three samples showed faint to moderate staining that exceeded the level seen in the normal samples, and the third was strongly positive in greater than 90% of the tumor cells.

These studies suggest that GPRA is upregulated in colon and breast cancer, and may be a potential marker or therapeutic target for these malignancies.

Methods

Antibody Titration and Study Protocol:

Antibody titration experiments were conducted with a Western positive affinity-purified anti-peptide antibody targeting the amino terminus of GPRA, to establish concentrations that would result in minimal background and maximal detection of signal. Serial dilutions were performed at concentrations of 20 ug/ml, 10 ug/ml, 5 ug/ml, and 2.5 ug/ml. The serial dilution study demonstrated the highest signal-to-noise ratio at a concentration of 2.5 ug/ml on paraffin-embedded, formalin-fixed tissues. This concentration was used for the current study. The antibody to GPRA 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.

Tissues were also stained with positive control antibodies (CD31 and vimentin) to ensure that the tissue antigens were preserved and accessible for immunohistochemical 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.

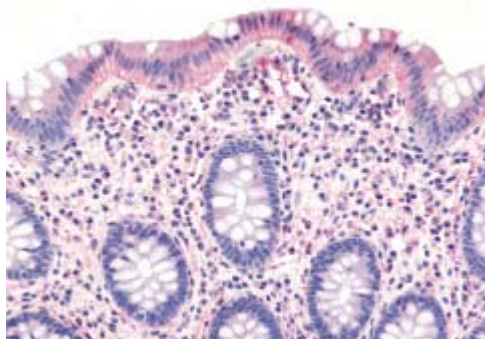
Slides were imaged with a DVC 1310C digital camera coupled to a Nikon microscope. Images were stored as TIFF files with Adobe Photoshop.

Results:

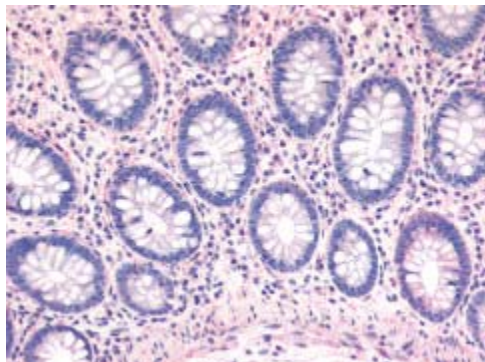
Colon, Normal

Sample 1: This sample of normal colon was obtained at surgery from a 65-year-old female.

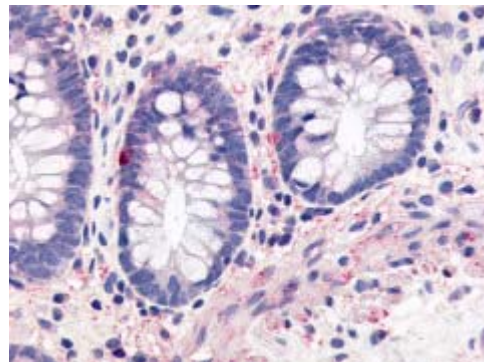
The epithelium of the superficial colonic mucosa showed blush staining. Enterocytes in the crypts were negative. Ganglion cells were negative. Neuroendocrine cells stained faintly. Lymphocytes and plasma cells within the lamina propria stained faintly. Smooth muscle cells of the muscularis mucosa, muscularis propria, and vascular smooth muscle showed blush staining, and endothelium was negative.



001: Surface Epithelium and Crypts 20X



002: Crypts 20X

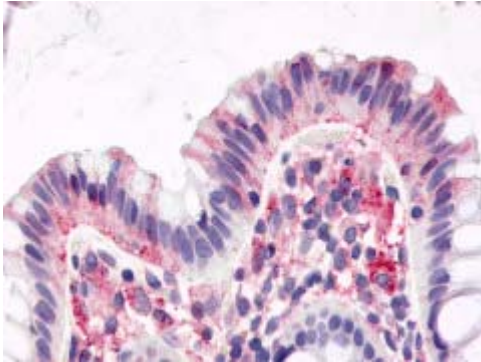


003: Crypt Epithelium with Neuroendocrine Cells 40X

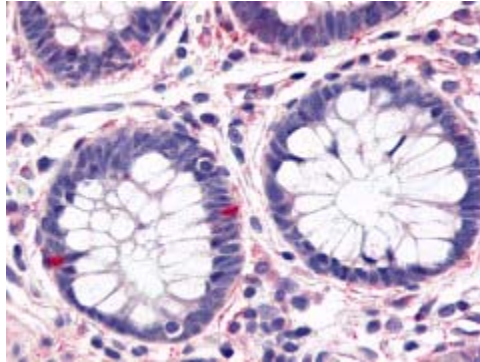
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Sample 2: This sample of normal colon was obtained from a 57-year-old male.

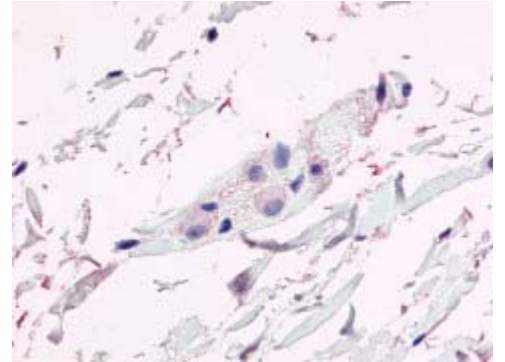
The epithelium of the superficial colonic mucosa showed blush to faint staining. Enterocytes in the crypts were negative. Ganglion cells showed blush staining. Neuroendocrine cells stained moderately. Lymphocytes showed blush to faint staining, and plasma cells within the lamina propria stained faintly. Smooth muscle cells of the muscularis mucosa and muscularis propria and vascular smooth muscle showed blush staining, and endothelium was negative.



004: Surface Epithelium 40X



005: Crypt Epithelium with Neuroendocrine Cells 40X

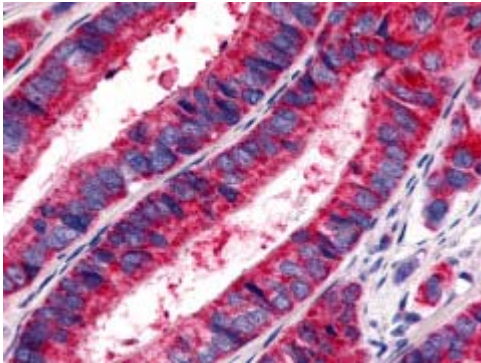


006: Submucosal Plexus 40X

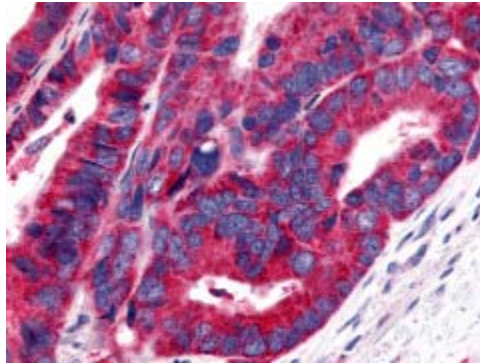
Colon, Adenocarcinoma

Sample 1: This sample of colon was obtained at surgery from an 81-year-old female.

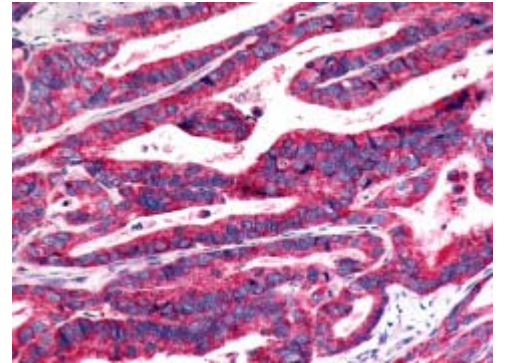
This sample of colon carcinoma was strongly positive in greater than 90% of carcinoma cells.



007: Adenomatous Epithelium 40X



008: Adenomatous Epithelium 40X

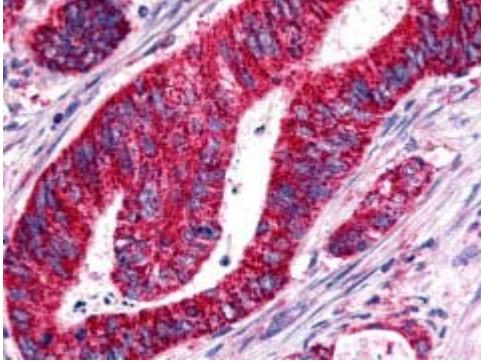


009: Ductal Adenocarcinoma 40X

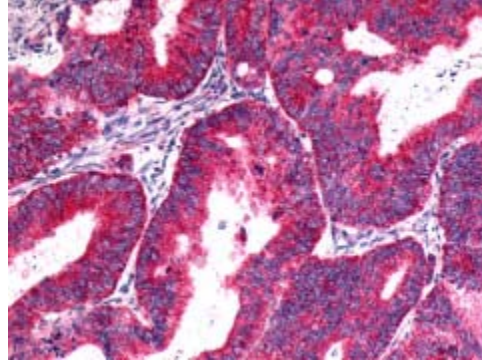
Sample 2: This sample of colon was obtained at surgery from a 60-year-old female.

This sample of colon carcinoma was strongly positive in greater than 90% of carcinoma cells.

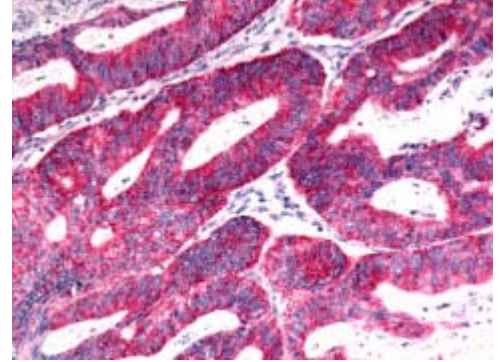
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010: Adenomatous Epithelium 40X

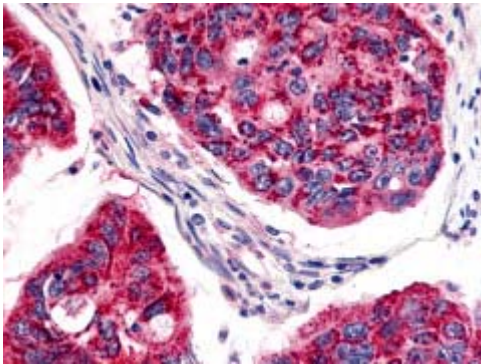


011: Ductal Adenocarcinoma 20X

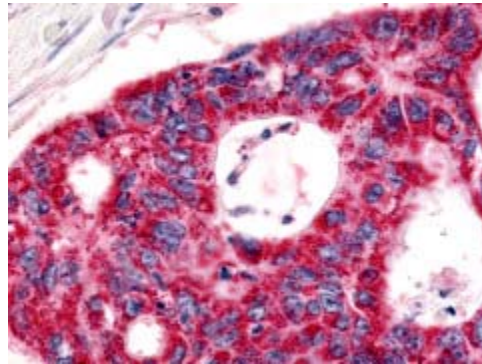


012: Ductal Adenocarcinoma 20X

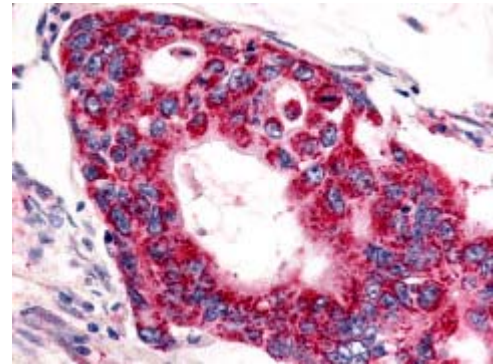
Sample 3: This sample of colon was obtained at surgery from a 77-year-old male. This sample of colon carcinoma was strongly positive in greater than 90% of carcinoma cells.



013: Neoplastic Cells and Fibroblasts 40X



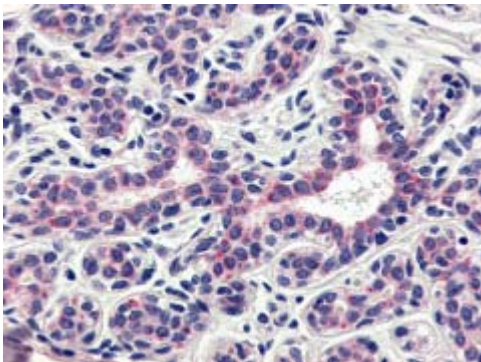
014: Ductal Adenocarcinoma 40X



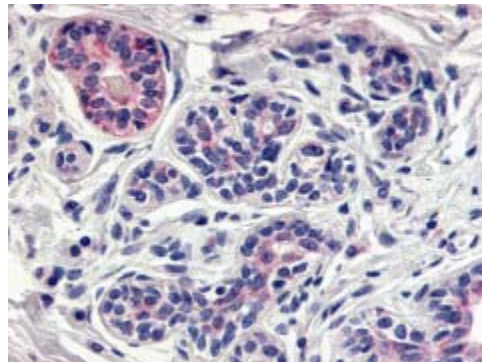
015: Ductal Adenocarcinoma 40X

Normal Breast

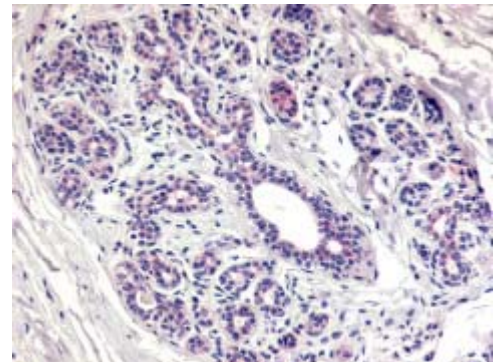
Sample 1: This sample of normal breast was obtained from a 32-year-old female who died of uremia. Lobular epithelium showed negative to faint staining. Ductal epithelium showed blush staining. Myoepithelial cells were negative or showed occasional blush staining.



016: Lobular and Ductal Epithelium 40X



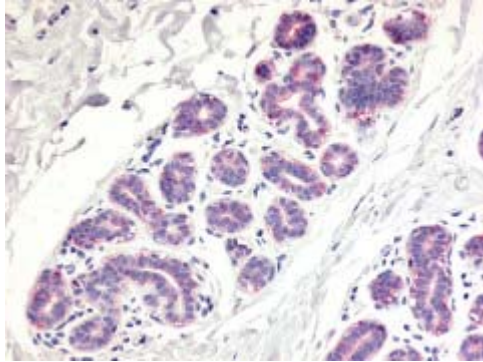
017: Lobular and Ductal Epithelium 40X



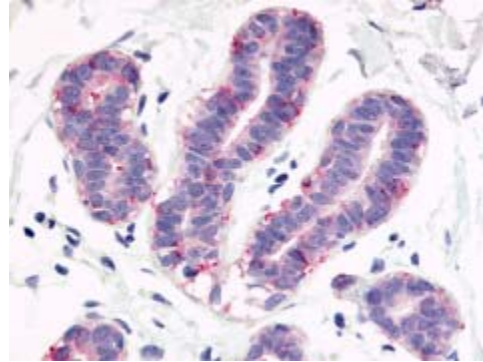
018: Lobule and Duct 20X

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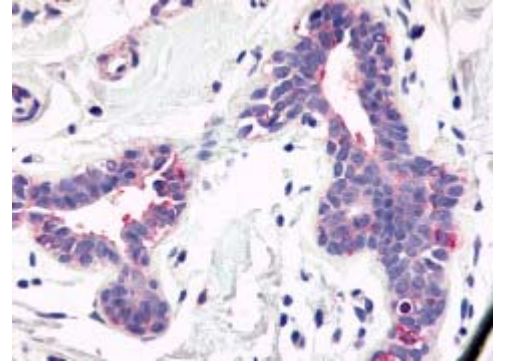
Sample 2: This sample of normal breast was obtained at breast excision from an 18-year-old female. Lobular epithelium showed blush to faint staining. Ductal epithelium showed blush staining. Myoepithelial cells were negative or showed blush staining.



019: Lobules 20X



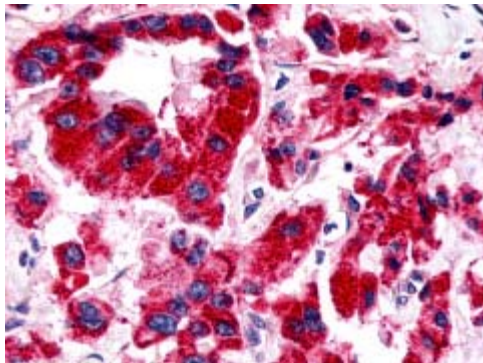
020: Lobular Epithelium 40X



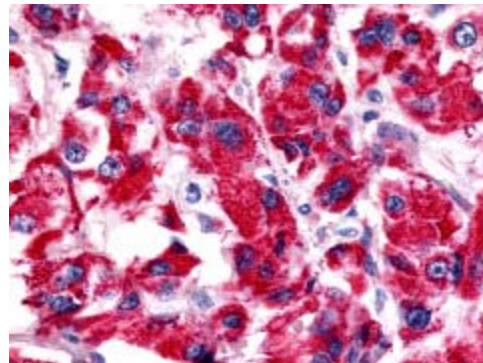
021: Ducts 40X

Breast, Adenocarcinoma (Infiltrating Ductal Carcinoma)

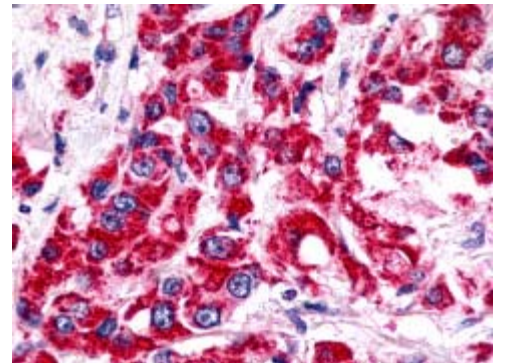
Sample 1: This sample of breast was obtained at surgery from a 75-year-old female. The sample of infiltrating ductal breast carcinoma showed strong cytoplasmic and membranous staining in greater than 90% of the carcinoma cells.



022: Infiltrating Carcinoma 40X



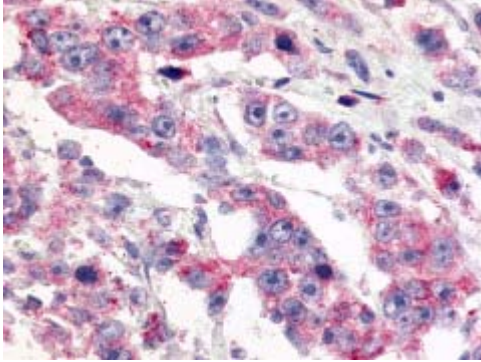
023: Infiltrating Carcinoma 40X



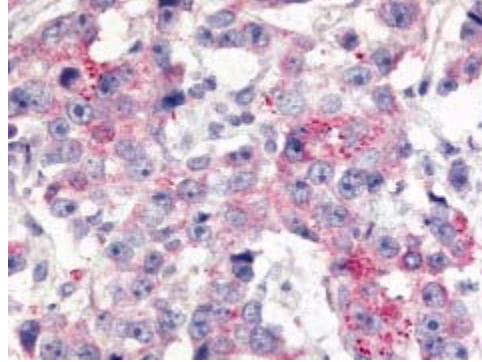
024: Infiltrating Carcinoma 40X

Sample 2: This sample of breast was obtained at surgery from a 53-year-old female. The sample of infiltrating ductal breast carcinoma showed moderate granular cytoplasmic and membranous staining in greater than 75% of the carcinoma cells.

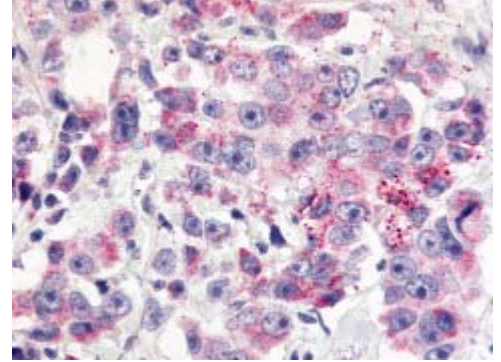
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025: Neoplastic Cells 40X

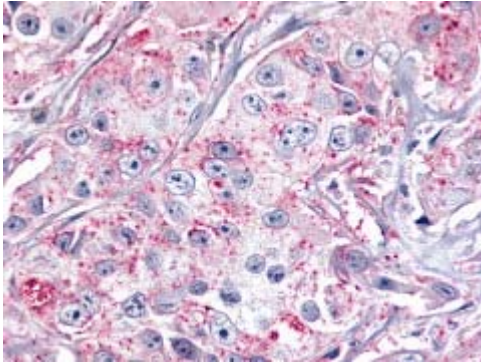


026: Neoplastic Cells 40X

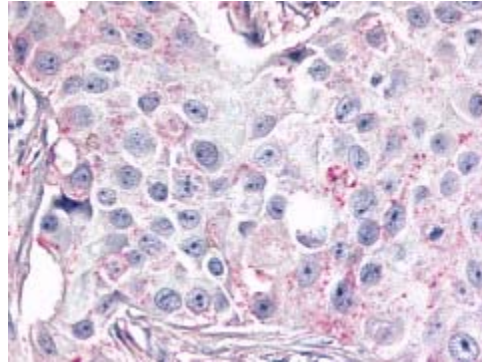


027: Neoplastic Cells 40X

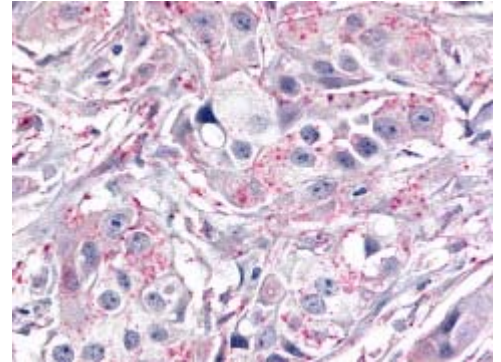
Sample 3: This sample of breast was obtained at surgery from a 72-year-old female. The sample of infiltrating ductal breast carcinoma showed faint to moderate granular cytoplasmic and membranous staining in greater than 75% of the carcinoma cells.



028: Infiltrating Carcinoma 40X



029: Infiltrating Carcinoma Cells 40X



030: Infiltrating Carcinoma 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.