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Sample Immunohistochemistry Co-localization Report for Antibody LPKinase-4 and Insulin, Glucagon and Somatostatin in Pancreatic Islets of Langerhans

Summary

Co-localization experiments were performed with Antibody LP-Kinase4 plus antibodies to insulin, glucagon, and somatostatin, in order to determine if this antibody was co-localizing with a specific subset of neuroendocrine cells in the islets of Langerhans. A subset of islet cells as well as neuroendocrine cells located within acini and adjacent to ducts co-localized with antibodies to LP-Kinase4 and somatostatin. There was no evidence of colocalized staining with antibodies to either insulin or glucagon.

Methods

Antibody Titration Protocol and Positive Control Study Results:

Antibody titration experiments were conducted with antibody LP-Kinase4 to establish concentrations that would result in minimal background and maximal detection of signal. Serial dilutions were performed at 2.5 ug/ml, 5 ug/ml, 10 ug/ml, and 20 ug/ml. The serial dilution study demonstrated the highest signal-to-noise ratio at concentrations of 10 ug/ml and 20 ug/ml on paraffin-embedded, formalin-fixed tissues. These concentrations were selected for the study. Antibody LP-Kinase4 was used as the primary antibody, and the principal detection system consisted of a Vector anti-rabbit secondary (BA-1000), and a Vector ABC-AP kit (AK-5000) with a Vector Red substrate kit (SK-5100), which produced a fuchsia-colored deposit.

In addition to staining with LP-Kinase4 alone, a double labeling experiment was performed with antibodies to glucagon (DAKO catalog# A0565, rabbit polyclonal), insulin (DAKO catalog #A564, guinea pig polyclonal), or somatostatin (DAKO catalog #A0566, rabbit polyclonal). Antibody titration experiments were conducted to establish dilutions that would result in minimal background and maximal detection of signal. Serial dilutions were performed at 1:500, 1:1000, and 1:2000 for the glucagon antibody, 1:250, 1:500, and 1:1000 for the insulin antibody, and 1: 2000, 1:4000 and 1:8000 for the somatostatin antibody on formalin-fixed, paraffin-embedded tissues. The dilution of 1:1000 was selected for antibodies to glucagon and insulin, and 1:8000 for somatostatin. These antibodies were used as the primary antibody, and the principle detection system consisted of a DAKO LSAB2 kit (catalog #K0675) and a DAKO DAB+ Chromogen-substrate (catalog #K3468). Development of a brown precipitate indicated interaction of the antibody with a target tissue or cells.

Tissues were also stained with positive control antibodies (CD31and 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 this 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.

Pancreas

Sample 1: This sample of normal pancreas was obtained from a 63-year-old female who died of atherosclerotic cardiovascular disease. The H&E stained sections showed pancreatic tissue with no diagnostic alterations.

Antibody to LP-Kinase4 (Vector Red) showed moderate staining in a subset of cells within pancreatic islets. Acinar epithelium and pancreatic ducts showed blush staining. Fibroblasts, leukocytes, adipocytes, peripheral nerves, endothelium, and vascular smooth muscle were negative.

Antibody to insulin (DAB brown) showed moderate staining in a subset of centrally clustered cells within pancreatic islets. Acinar and ductal epithelium, adipocytes, fibroblasts, endothelium, peripheral nerves, leukocytes, and vascular smooth muscle were negative.

Combined staining with antibody to LP-Kinase4 and insulin resulted in no evidence of colocalization. Instead, distinct single

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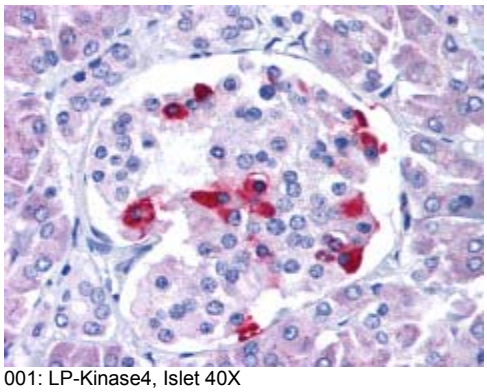
labeling of separate islet cell subsets for LP-Kinase4 (red) and insulin (brown) was present, as well as a third subset of islet cells that were negative.

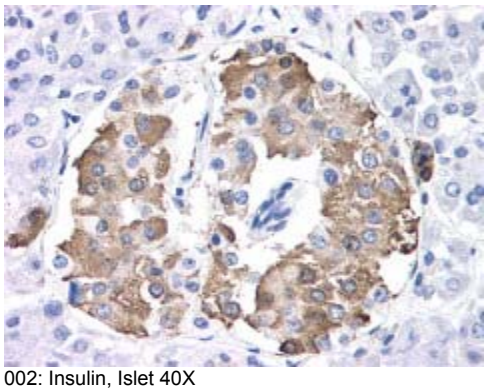
Antibody to glucagon alone (DAB brown), showed moderate staining in a small subset of peripherally distributed cells within pancreatic islets. Acinar and ductal epithelium, adipocytes, fibroblasts, endothelium, peripheral nerves, leukocytes, and vascular smooth muscle were negative.

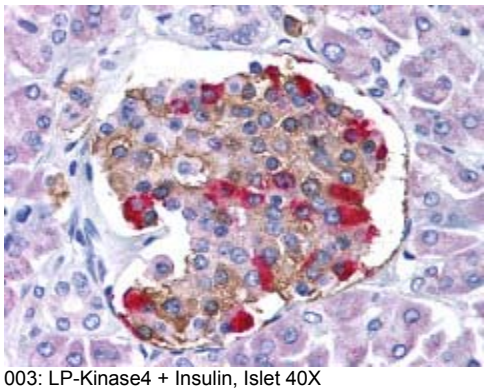
Combined staining with antibody to LP-Kinase4 and to glucagon resulted in no evidence of colocalized staining. Instead, distinct single labeling of separate islet cell subsets for LP-Kinase4 (red) and glucagon (brown) was present, as well as a third subset of islet cells that showed only minimal blush staining. The remaining cell types were negative, including pancreatic ducts, fibroblasts, adipocytes, peripheral nerves, endothelium, leukocytes, and vascular smooth muscle.

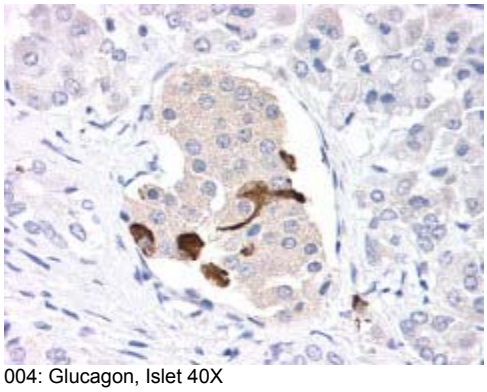
Antibody to somatostatin alone (DAB brown), showed moderate staining in a subset cells within pancreatic islets. Acinar and ductal epithelium, adipocytes, fibroblasts, endothelium, peripheral nerves, leukocytes, and vascular smooth muscle were negative.

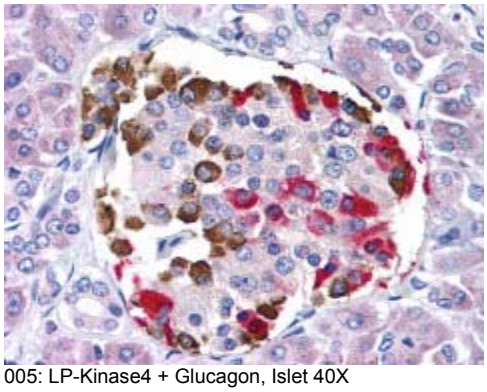
Combined staining with antibody to LP-Kinase4 and to somatostatin resulted in colocalized staining (brownish-red hue) in a subset of pancreatic islet cells. Acinar and ductal epithelium showed minimal blush LP-Kinase4 signal. The remaining cell types were negative, including fibroblasts, adipocytes, peripheral nerves, endothelium, leukocytes, and vascular smooth muscle.


001: LP-Kinase4, Islet 40X


002: Insulin, Islet 40X


003: LP-Kinase4 + Insulin, Islet 40X


004: Glucagon, Islet 40X


005: LP-Kinase4 + Glucagon, Islet 40X

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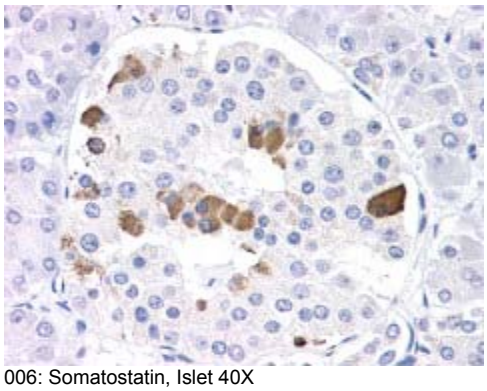
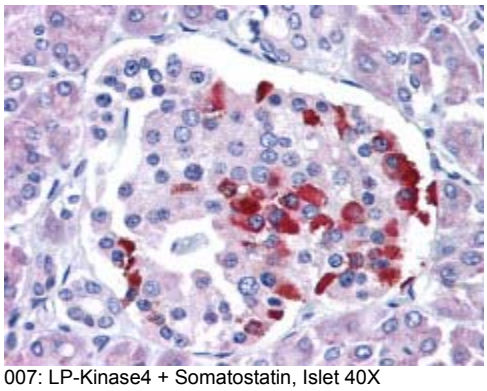


006: Somatostatin, Islet 40X



007: LP-Kinase4 + Somatostatin, Islet 40X

Sample 2: This sample of normal pancreas was obtained from a 72-year-old female who died of congestive heart failure. The H&E stained sections showed unremarkable pancreatic tissue.

Antibody to LP-Kinase4 (Vector Red) showed moderate staining in a subset of randomly distributed cells within pancreatic islets. Acinar epithelium showed blush to faint staining, and fibroblasts, leukocytes, adipocytes, peripheral nerves, endothelium, and vascular smooth muscle were negative.

Antibody to insulin (DAB brown), showed moderate staining in a large subset of centrally clustered cells within pancreatic islets. Acinar and ductal epithelium, adipocytes, fibroblasts, endothelium, peripheral nerves, leukocytes, and vascular smooth muscle were negative.

Combined staining with antibody to LP-Kinase4 plus antibody to insulin resulted in no evidence of colocalization. Instead, distinct single labeling of separate islet cell subsets for LP-Kinase4 (red) and insulin (brown) was present, as well as a third subset of islet cells that was mostly negative. Acini and ducts showed minimal blush (pink) staining. The remaining cell types were negative, including fibroblasts, adipocytes, peripheral nerves, leukocytes, endothelium, and vascular smooth muscle.

Antibody to glucagon alone (DAB brown), showed moderate staining in a small subset of peripherally distributed cells within pancreatic islets. Minimal blush staining was present in the remaining islet cells and acinar epithelium. Ductal epithelium, adipocytes, fibroblasts, endothelium, peripheral nerves, leukocytes, and vascular smooth muscle were negative.

Combined staining with antibody to LP-Kinase4 and glucagon resulted in no evidence of colocalized staining. Instead, distinct single labeling of separate islet cell subsets for LP-Kinase4 (red) and glucagon (brown) was present, as well as a third subset of islet cells that showed only minimal blush staining. The remaining cell types were negative, including pancreatic ducts, acini, fibroblasts, adipocytes, peripheral nerves, endothelium, leukocytes, and vascular smooth muscle.

Antibody to somatostatin alone (DAB brown), showed moderate staining in a subset of cells within pancreatic islets. The remaining cell types were negative, including acinar epithelium, ductal epithelium, adipocytes, fibroblasts, endothelium, peripheral nerves, leukocytes, and vascular smooth muscle.

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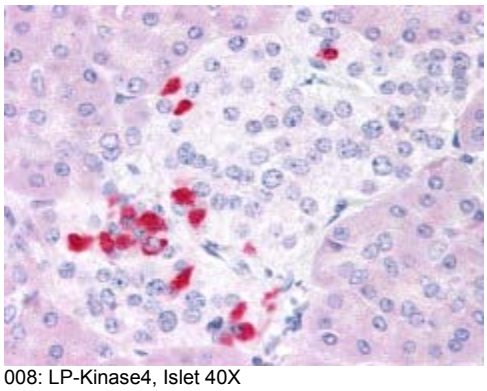
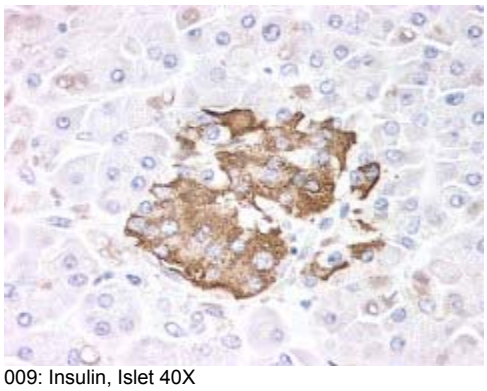
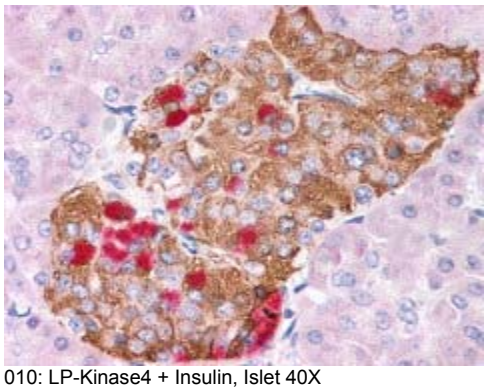
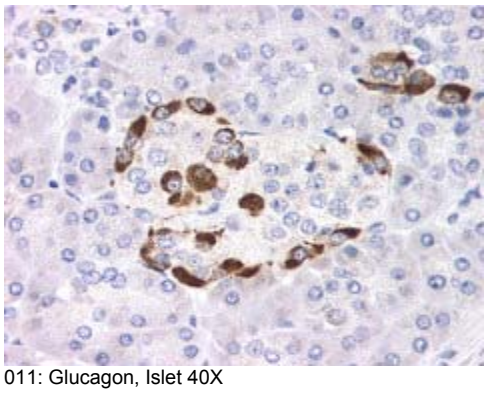
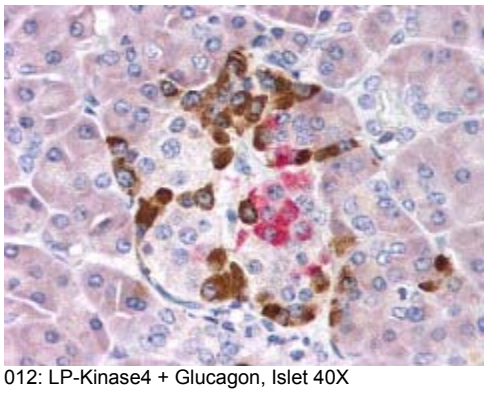
Combined staining with antibody to LP-Kinase4 and antibody to somatostatin resulted in co-localized staining (brownish-red hue) in a subset of pancreatic islet cells. The remaining cell types were negative, including pancreatic ducts, acini, fibroblasts, adipocytes, peripheral nerves, endothelium, leukocytes, and vascular smooth muscle.

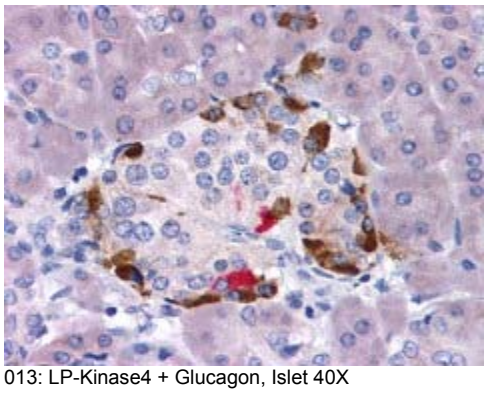

008: LP-Kinase4, Islet 40X

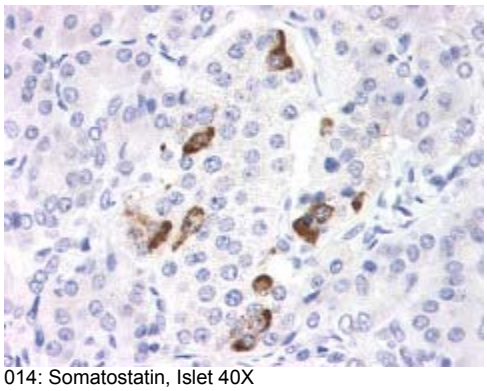

009: Insulin, Islet 40X

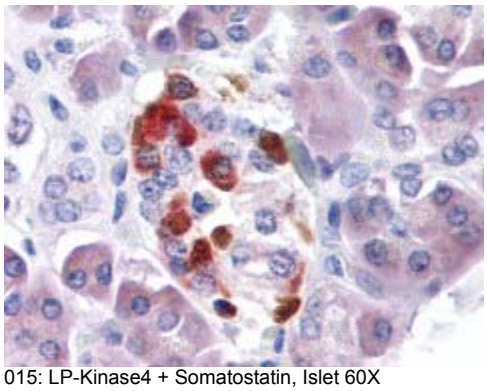

010: LP-Kinase4 + Insulin, Islet 40X

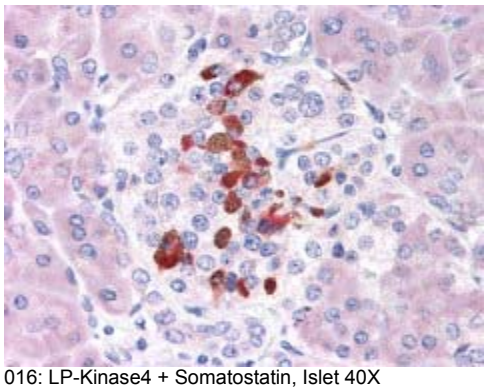

011: Glucagon, Islet 40X


012: LP-Kinase4 + Glucagon, Islet 40X


013: LP-Kinase4 + Glucagon, Islet 40X


014: Somatostatin, Islet 40X


015: LP-Kinase4 + Somatostatin, Islet 60X


016: LP-Kinase4 + Somatostatin, Islet 40X

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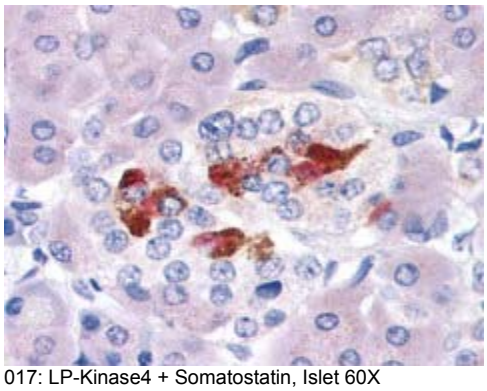
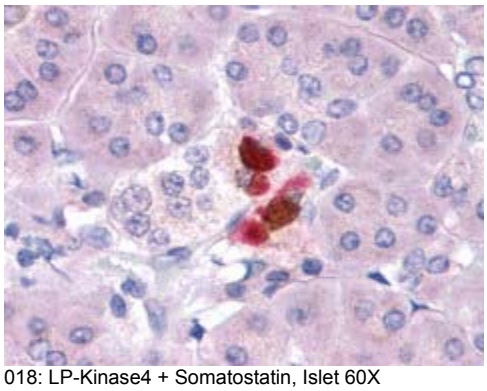


017: LP-Kinase4 + Somatostatin, Islet 60X



018: LP-Kinase4 + Somatostatin, Islet 60X

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.

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