

## Antibody LP-BR2 Immunohistochemistry Report on Atherosclerosis and Myocardial Infarct

### Summary

Antibody LP-BR2, a Western positive rabbit polyclonal antibody targeting the amino terminus of the BR2 protein, was evaluated in immunohistochemistry on samples of normal artery, atherosclerotic artery, normal heart, and acute myocardial infarct.

Compared to normal artery samples, there was significantly increased staining of medial smooth muscle in areas of atherosclerosis, and increased staining of myointimal cells and foamy macrophages. Within the myocardial infarct samples, there was significantly increased staining within myocytes adjacent to areas of necrosis and increased staining of endothelial cells in areas of injury. These studies suggest that BR2 is upregulated in atherosclerosis and myocardial injury.

### 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 BR2, 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 BR2 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.

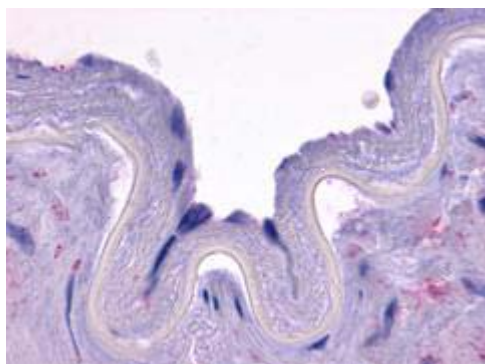
Slides were imaged with a DVC 1310C digital camera coupled to a Nikon microscope. Images were stored as TIFF files with Adobe Photoshop.

### Results:

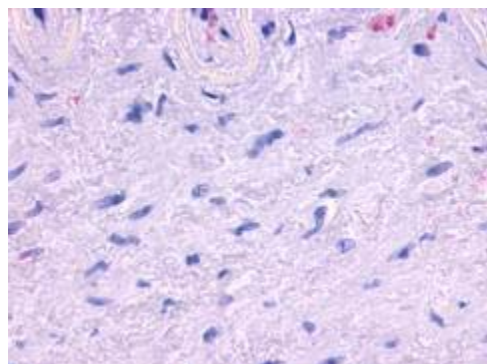
#### Artery

**Sample 1:** This sample of normal artery was obtained at surgery from a 62-year-old male.

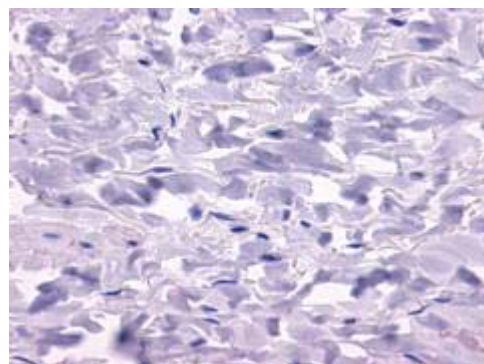
Intimal endothelium was negative. Myointimal cells, fibroblasts, and smooth muscle cells were negative.



001: Artery, Endothelium and Internal Elastic Lamina  
60X



002: Artery, Tunica Media 40X

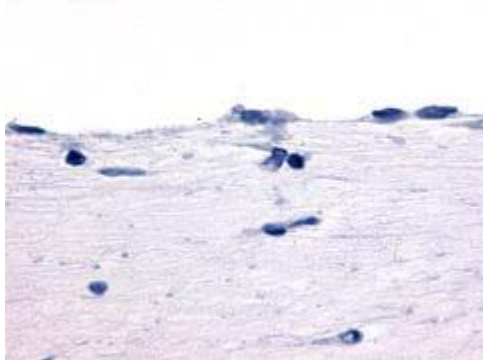


003: Artery, Tunica Adventitia 40X

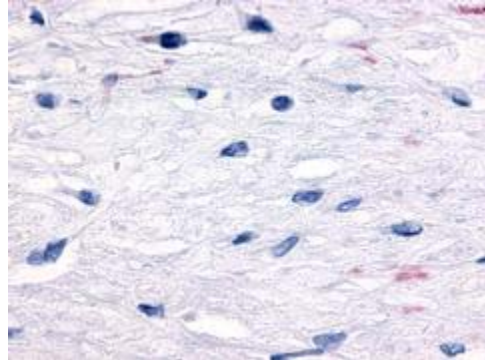
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**Sample 2:** This sample of normal artery was obtained from a 16-year-old male who died of trauma.

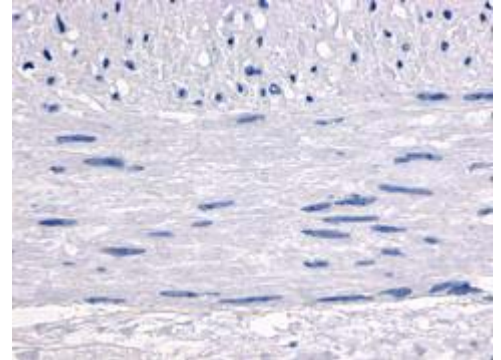
Intimal endothelium was negative. Myointimal cells, fibroblasts, and smooth muscle cells were negative.



004: Artery, Intimal Endothelium and Myointimal Cells 60X



005: Artery, Myointimal Cells 60X

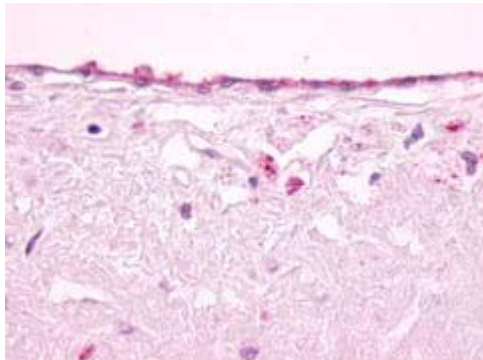


006: Artery, Smooth Muscle of Tunica Media 40X

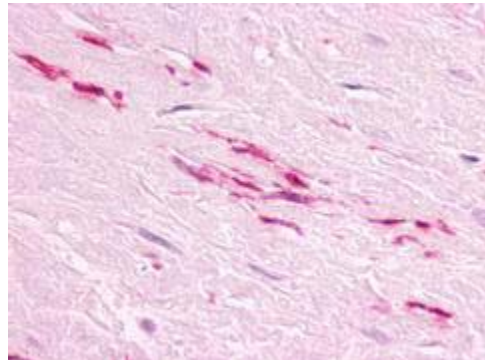
### Artery, Atherosclerosis

**Sample 1:** This sample of atherosclerotic artery was obtained at autopsy from a 59-year-old female.

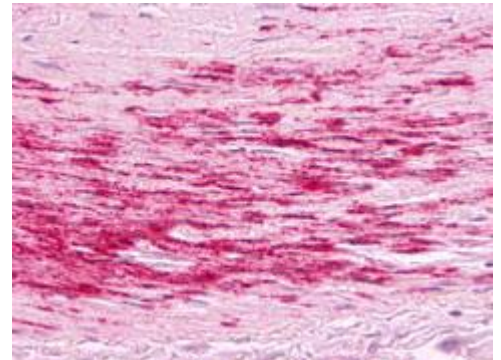
In areas showing moderate to severe atherosclerosis, endothelium was moderately positive. Myointimal cells within the atheroma were moderately positive, and foamy macrophages were moderately positive. Within atherosclerotic vessels, very strong staining was seen within areas of medial smooth muscle, particularly underlying or adjacent to plaques. Staining was significantly less in areas with more normal arterial architecture and minimal atheroma formation. Within the tunica adventitia, fibroblasts were negative, and within the vasa vasorum, endothelial cells were faintly to moderately positive and smooth muscle cells were moderately positive. Lymphocytes, neutrophils, and plasma cells were negative. Adipocytes were negative, Schwann cells were negative, and fibroblasts were negative. Compared to normal arteries, samples of atherosclerotic artery showed significantly increased staining within smooth muscle cells in areas of atherosclerosis, and increased staining within myointimal cells and foamy macrophages.



007: Artery, Atherosclerosis, Intimal Endothelium 60X



008: Artery, Atherosclerosis, Myointimal Cells 60X



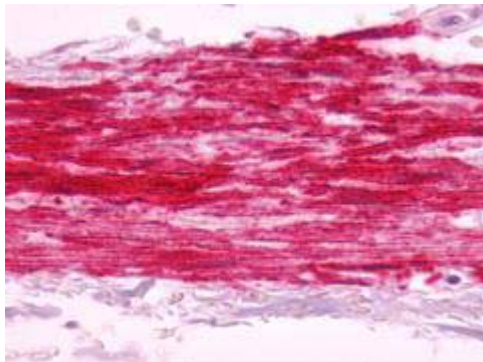
009: Artery, Atherosclerosis, Tunica Media Underlying Area of Plaque 40X

**Sample 2:** This sample of atherosclerotic artery was obtained at autopsy from a 66-year-old male.

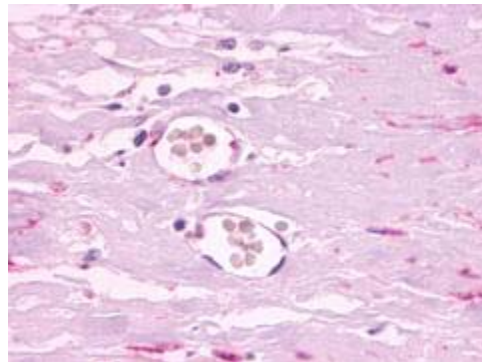
In areas showing moderate to severe atherosclerosis, endothelium was moderately positive. Myointimal cells within the atheroma and foamy macrophages were moderately positive. Within atherosclerotic vessels, very strong staining was seen

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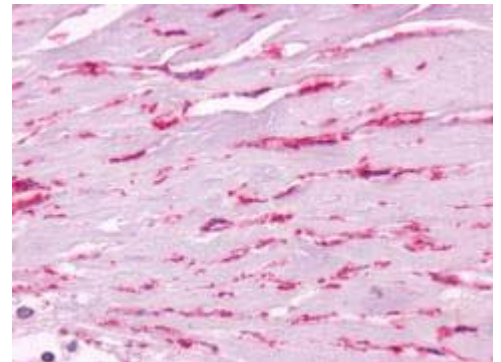
within areas of medial smooth muscle, particularly underlying or adjacent to plaques. Staining was less in areas with normal arterial architecture or minimal atheroma formation. Within areas of neovascularization, endothelial cells lining capillaries were faintly positive. Within the tunica adventitia, fibroblasts were negative, and within the vasa vasorum, endothelial cells were faintly to moderately positive and smooth muscle cells were faintly to strongly positive. Lymphocytes, neutrophils, and plasma cells were negative. Adipocytes, Schwann cells, and mesenchymal fibroblasts were negative. Compared to normal arteries, samples of atherosclerotic artery showed significantly increased staining within smooth muscle cells in areas of atherosclerosis, and increased staining within myointimal cells and foamy macrophages.



010: Artery, Atherosclerosis, Tunica Media 40X



011: Artery, Atherosclerosis, Area of Neovascularization 60X

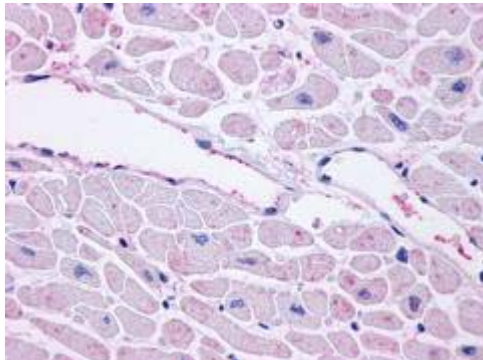


012: Artery, Atherosclerosis, Myointimal Cells and Foamy Macrophages 60X

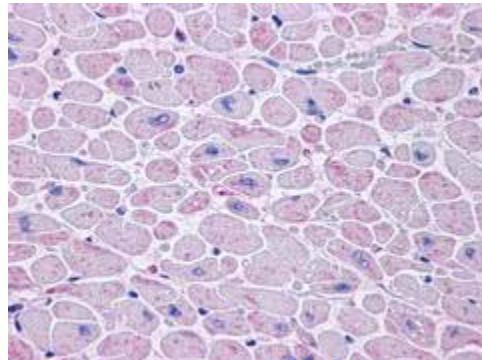
### Heart

**Sample 1:** This sample of normal heart was obtained at autopsy from a 69-year-old male.

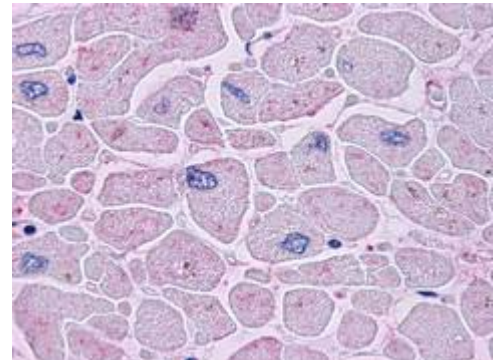
Cardiac myocytes were negative to faintly positive. Capillary and lymphatic endothelium showed occasional faintly positive cells. Interstitial fibroblasts were negative. Within muscular vessels, endothelium was faintly positive, and vascular smooth muscle was moderately positive. Adipocytes were negative.



013: Heart, Capillaries and Myocytes 40X



014: Heart, Myocytes 40X

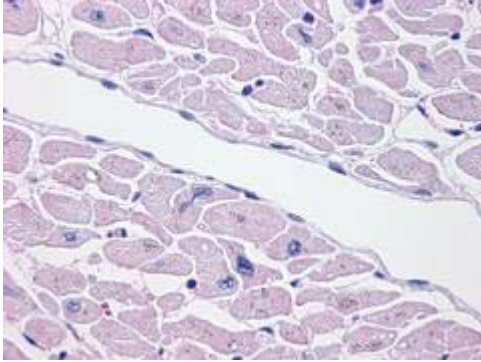


015: Heart, Myocytes 60X

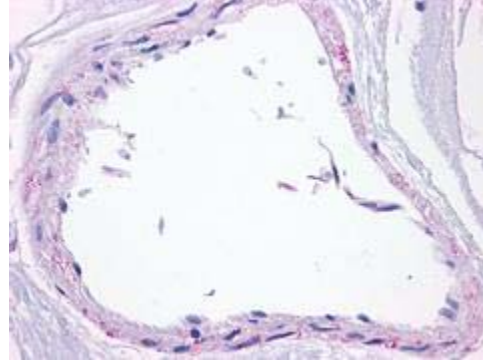
**Sample 2:** This sample of normal heart was obtained at autopsy from a 85-year-old female.

Cardiac myocytes were faintly positive. Capillary and lymphatic endothelium were occasionally faintly positive cells. Interstitial fibroblasts were negative. Within muscular vessels, endothelium was faintly positive, and vascular smooth muscle was moderately positive. Adipocytes were negative.

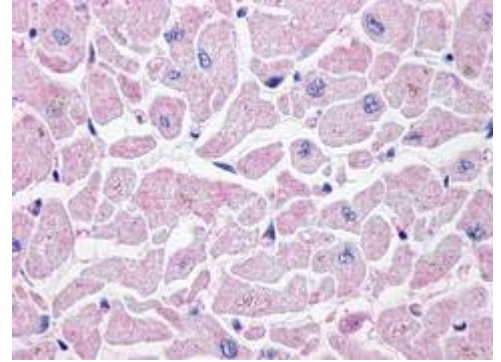
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016: Heart, Capillaries and Myocytes 40X



017: Heart, Vessel 40X

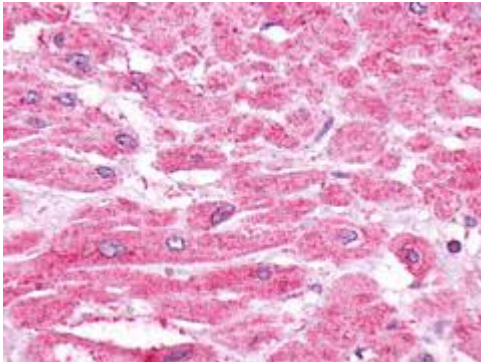


018: Heart, Myocytes 40X

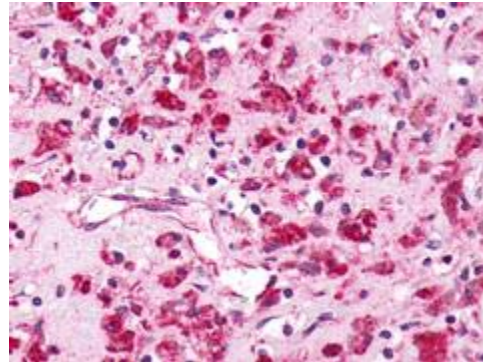
### Heart, Myocardial Infarct

**Sample 1:** This sample of heart with myocardial infarct was obtained at autopsy from a 62-year-old female.

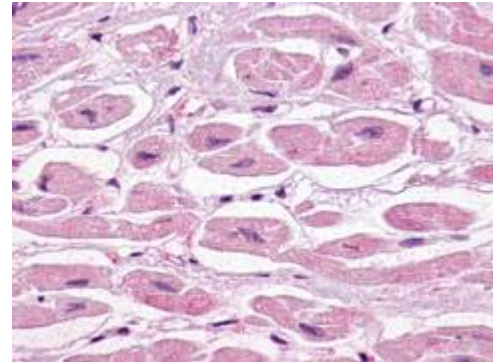
Cardiac myocytes in preserved areas were faintly positive. In areas of acute injury, cardiac myocytes adjacent to areas of necrosis were strongly positive, and those undergoing coagulative necrosis were negative. Endothelial cells in areas of injury were strongly positive, and staining appeared to be significantly increased within capillaries and small vessels within and adjacent to the injury focus. Neutrophils and macrophages were also strongly positive. Compared to normal heart samples, there was significantly increased staining in preserved myocytes adjacent to areas of necrosis, increased staining within endothelial cells in areas of injury and adjacent to injury.



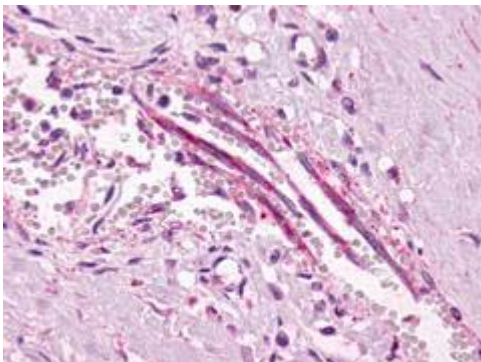
019: Heart, Myocardial Infarct, Cardiac Myocytes Adjacent to Area of Necrosis 40X



020: Heart, Myocardial Infarct, Macrophages and Vessels 40X



021: Heart, Myocardial Infarct, Preserved Cardiac Myocytes 40X

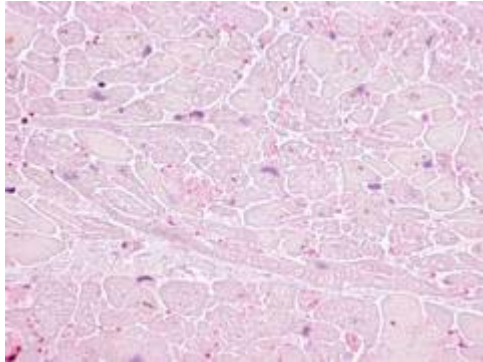


022: Heart, Myocardial Infarct, Vessels 40X

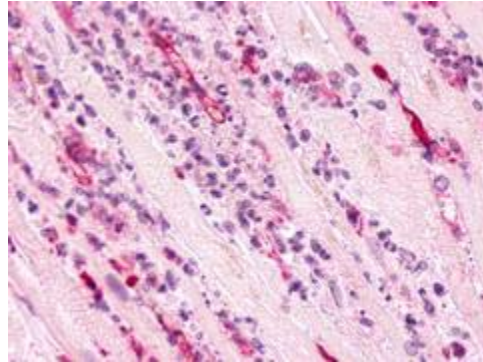
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**Sample 2:** This sample of heart with myocardial infarct was obtained at autopsy from a 61-year-old of unknown sex.

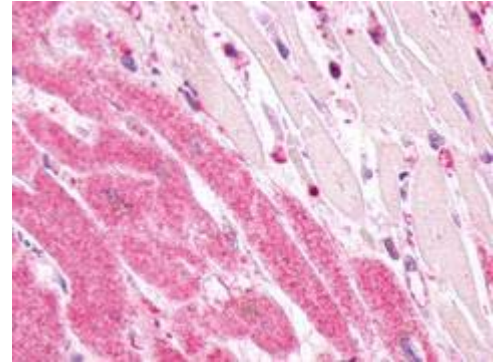
Cardiac myocytes in preserved areas were faintly positive. In areas of acute injury, cardiac myocytes adjacent to areas of necrosis were strongly positive, and those undergoing coagulative necrosis were negative. Endothelial cells in areas of injury were strongly positive, and staining appeared to be significantly increased within capillaries and small vessels within and adjacent to the injury focus. Neutrophils and macrophages were also strongly positive. Within one occluded vessel, there was strong positive staining of underlying tunica media smooth muscle and faint staining of the endothelial cells within the granulation tissue of the organizing thrombus. Compared to normal heart samples, there was significantly increased staining in preserved myocytes adjacent to areas of necrosis and increased staining within endothelial cells in areas of injury and adjacent to injury.



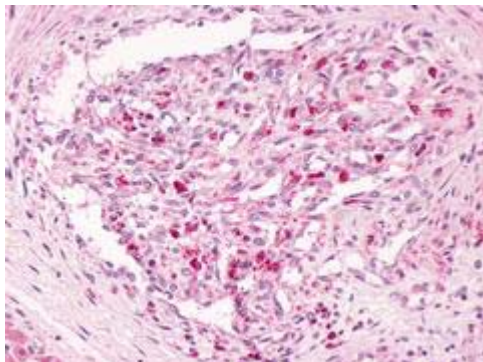
023: Heart, Myocardial Infarct, Area of Coagulative Necrosis 40X



024: Heart, Myocardial Infarct, Area of Infarct 40X



025: Heart, Myocardial Infarct, Infarct Boundary 40X



026: Heart, Myocardial Infarct, Occluded Vessel with Organizing Thrombus 20X

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.