ANTIBODY RESOURCE

ANTIBODY TESTING REPORT

SUMMARY

Antigen: Connexin 43 (Uniprot# P17302)

Method tested: Immunohistochemistry

Laboratory ID: LAB07

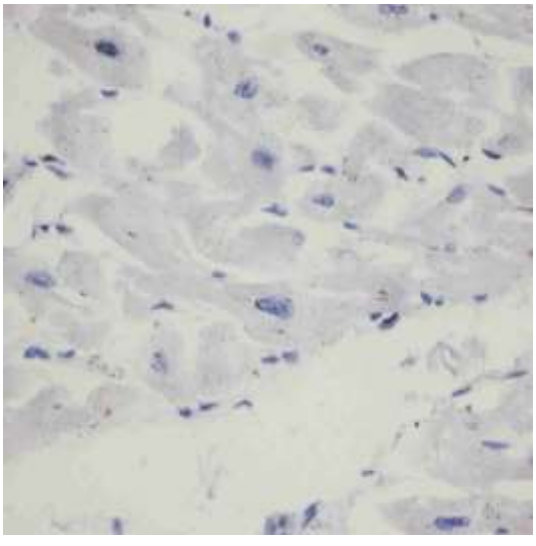
Project ID: AR162

With thousands of proteins and often hundreds of associated antibodies, the selection of a specific antibody can be both time-consuming and expensive. Antibody Resource is spearheading a unique initiative designed to compare antibodies from numerous suppliers using identical samples/tissues and an identical protocol. In doing so, we hope to enable scientists to form an unrivalled opinion of which is the most suitable antibody for their research and in particular, which is going to require the least amount of optimisation, a process which can often take weeks or months.

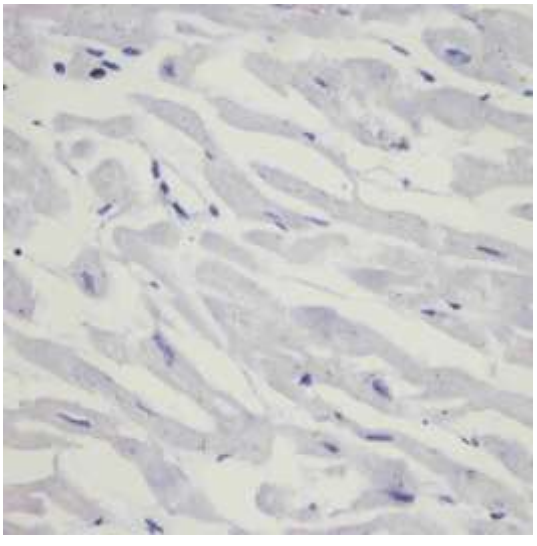
For the purposes of the antibody comparison initiative, we select the best antibodies from each manufacturer and then compare them side-by-side using the same experimental conditions to provide a direct comparison. The antibodies are collected centrally, repackaged and given an internal reference ID prior to delivery to independent laboratories to ensure objective testing and to minimise bias.

RESULTS

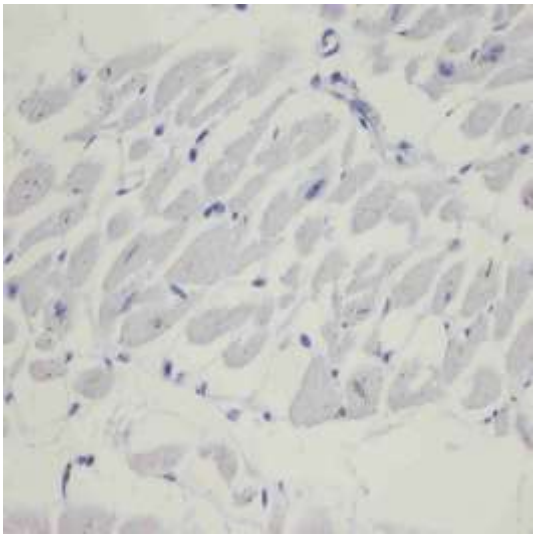
Immunohistochemical analysis of formalin fixed, paraffin embedded Human heart tissue using various anti-CX43 antibodies and isotype controls (see Method section for more detail).



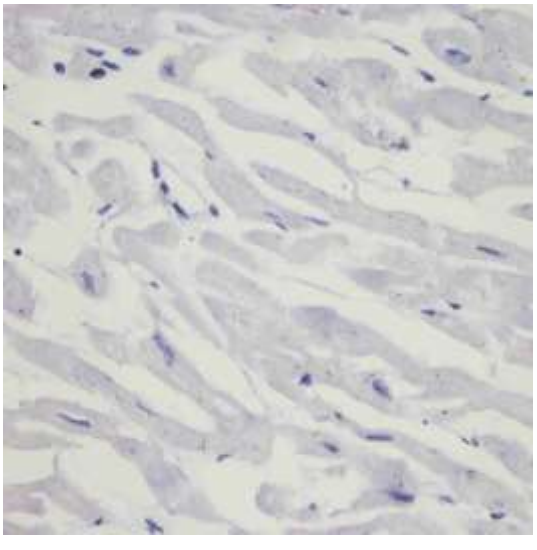
Antibody : CX43 P123 at 1/500



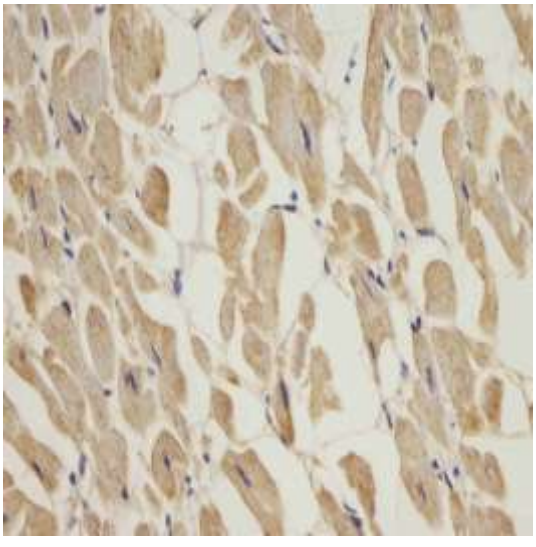
Antibody : Isotype control



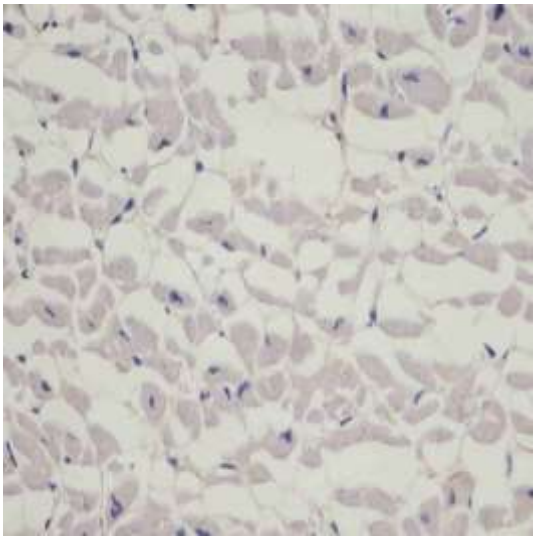
Antibody : CX43 P135 at 1/500



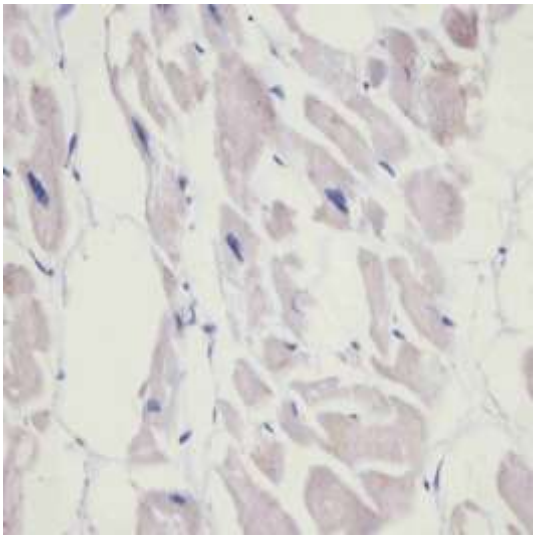
Antibody : Isotype control



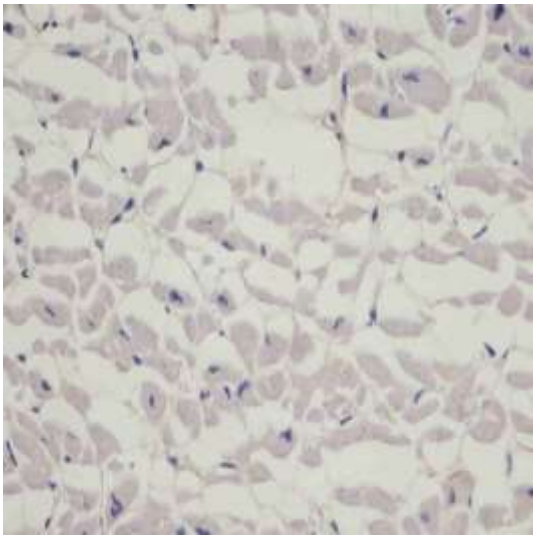
Antibody : CX43 P147 at 1/50



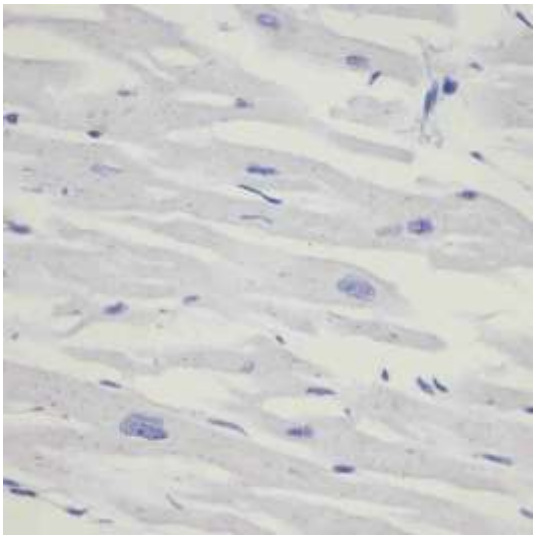
Antibody : Isotype control



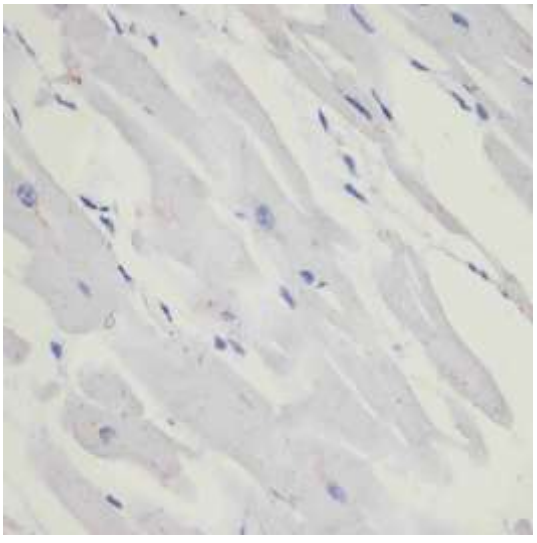
Antibody : CX43 P166 at 1/50



Antibody : Isotype control



Antibody : CX43 M183 at 1/50



Antibody : Isotype control

METHOD

Antibodies

Primary antibody	Secondary antibody	Isotype Control
CX43 P123 at 1/500 (Supplier 33)	Peroxidase-conjugated AffiniPure Bovine Anti-Goat IgG (H+L) (Jackson ImmunoResearch, 805-035-180) at 1/300	ChromPure Goat IgG,whole molecule (Jackson ImmunoResearch, 005-000-003) at 1/500
CX43 P135 at 1/500 (Supplier 33)	Peroxidase-conjugated AffiniPure Bovine Anti-Goat IgG (H+L) (Jackson ImmunoResearch, 805-035-180) at 1/300	ChromPure Goat IgG,whole molecule (Jackson ImmunoResearch, 005-000-003) at 1/500
CX43 P147 at 1/50 (USBiological)	HRP Anti-Polyvalent kit 'ready-to-use' (ScyTek Laboratories, UHP500)	Rabbit IgG Isotype Control (ThermoFisher Scientific, MA5-16385) at 1/50
CX43 P166 at 1/50 (Supplier 19)	HRP Anti-Polyvalent kit 'ready-to-use' (ScyTek Laboratories, UHP500)	Rabbit IgG Isotype Control (ThermoFisher Scientific, MA5-16385) at 1/50
CX43 M183 at 1/50 (Supplier 19)	HRP Anti-Polyvalent kit 'ready-to-use' (ScyTek Laboratories, UHP500)	Mouse IgG1 Isotype Control (ThermoFisher Scientific, SA1-12182) at 1/50

= Component of the CX43 Superstarter Antibody Panel. See end of report for details.

PROTOCOL

Immunhistochemical analysis of formalin fixed, paraffin embedded Human heart tissue was performed using Nikon's DS-Ri1 system.

1. Tissue slides were preheated in convection oven at 60°C for 30min**.**
2. Deparaffinization was performed by immersing the slides three times in xylene for 10 minutes each time, followed by 5 minutes in 100% ethanol; then 5 minutes in 95% ethanol, 5 minutes in 80% ethanol, 5 minutes in 70% ethanol and finally three washes in distilled water of 5 minutes per wash.
3. An antigen retrieval procedure was then performed by heating the tissue slides, immersed in 10mM sodium citrate buffer, pH 6.0, in a microwave for 8 – 15 minutes. The slices were then allowed to cool at room temperature for 20 – 30 minutes.
4. Endogenous peroxidases were blocked by soaking the slides in 3% hydrogen peroxide-methanol for 15 minutes at room temperature, followed by two washes in distilled water of 5 minutes per wash and one wash of 5 minutes in PBS. Blocking was completed by incubating the slices in 3% BSA in PBS for 30 minutes at room temperature.

5. The slides were then immersed in the primary antibody solution diluted in PBS containing 3% BSA at 37°C for 1 hour or overnight at 4°C in a humidified chamber. Each antibody was diluted according to the working range suggested by the supplier (for details see table above).
6. Following three washes for 5 minutes each wash at room temperature with PBS-Tween (PBST), the slides were incubated in secondary antibody. This was either in the biotinylated secondary antibody from the HRP Anti-Polyvalent kit for 30 minutes at 37°C in a humidified chamber or if the Peroxidase-conjugated AffiniPure Bovine Anti-Goat IgG was used, the slide was incubated for 60 minutes at 37°C in a humidified chamber (for details see table above).
7. After removal of the secondary antibody solution, the slides were washed three times for 5 minutes per wash in PBST and then incubated in Streptavidin-HRP solution for 10 minutes at 37°C if the HRP Polyvalent kit was used or on to step 8 if the Peroxidase-conjugated AffiniPure Bovine Anti-Goat IgG was used as the secondary antibody.
8. DAB staining solution was immediately added and the slides observed until the desired colour change was obtained (typically between 30 seconds and 5 minutes). After draining away excess solution, the sides were placed into distilled water for 5 minutes.
9. The slides were then incubated with haematoxylin for 3 minutes as counterstain.
10. Following three washes with distilled water, the slides were dehydrated by subsequent 5 minute washes in 70% ethanol, 80% ethanol, 95% ethanol, twice with 100% ethanol and two 10 minute washes with xylene. A coverslip was secured on each slide
11. The resulting staining of the tissue was observed and recorded.

EXPERIMENTAL NOTES

Under these experimental conditions, CX43 P147 exhibits membrane and cytoplasm staining in the Human heart tissue which is consistent with the expected locations. No staining is seen using CX43 P123, CX43 P135, CX43 P166 or CX43 M183. These antibodies could be further investigated at higher concentrations.

SUPERSTARTER ANTIBODY PANELS

A panel of Superstar antibodies in trial sizes, to enable you to economically test the best antibodies, to determine which is going to be the best for your research project for only $307, €267, £198.

The Connexin 43 Superstarter Antibody Panel consists of:

1 x 10 ug	71-0700	(Invitrogen Antibodies)
1 x 10 ug	AB1728	(Millipore)
1 x 25 ul	C6219	(Sigma-Aldrich)
1 x 25 ul	AB0016-200	(SICGEN)

http://www.antibodyresource.com/superstars

Images of Superstar Connexin 43 antibodies: