VERSION 1

JUL 12, 2019

Pig- Heart Neuonal and Fiber Immunocytochemistry V.1

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OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.2n9gdh6

Protocol Citation: Pradeep Rajendran, John Tompkins, Kalyanam Shivkumar 2019. Pig- Heart Neuonal and Fiber Immunocytochemistry.

protocols.io

<https://dx.doi.org/10.17504/protocols.io.2n9gdh6>

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Protocol status: Working
We use this protocol and it's working

Created: May 07, 2019

Last Modified: Jul 12, 2019

PROTOCOL integer ID:
22977

1 Animal: Yucatan Minipig 1427

-Gender: Male
-Weight: 28.4Kg

2 Tissue Fixation:

-Flush heart with PBS, RT, and heparin then inject 4% paraformaldehyde in PBS into coronaries.
-Removed heart and fix overnight at 4 degrees C in 4% PFA/PBS.
-Transfer to container with 0.01M PBS+0.02M Na Azide+20% Sucrose on 6/15/2018..

3 Tissue Processing:

-Cut the right atrial ganglionated plexus (RAGP) out of heart.
-Rinsed tissue with 1XPBS, then froze in (-80) freezer.
- Used O.C.T medium to mount RAGP on specimen plate in cryostat.
-Cut RAGP at 30um at (-25) degrees Celsius.
-9 slides x 8 sets
-2 tissue sections per slide
-Make sure the cut sections have completely adhered to the slides (approx 30 min) before wrapping the slide boxes in foil and placing in (-20) freezer.

4 Fluorescence Immunohistochemistry:

Primary Antibodies:

1.) VACHT

-Synaptic Systems (SYSY): Cat.No.139103

-Dilution: (1:500)

-Host: rabbit

2.) Anti-Tyrosine Hydroxylase

-Millipore: Cat. #AB1542

-Dilution: (1:500)

-Host: sheep

5 Secondary Antibodies:

1.) Alexa Fluor 488-conjugated AffiniPure Donkey Anti-Rabbit IgG

-Jackson ImmunoResearch Lab: Code No. 711-545-152

-Dilution: (1:200)

2.) Biotin-SP-conjugated AffiniPure Donkey Anti-Goat IgG

-Jackson ImmunoResearch Lab: Code No.705-065-147

-Dilution: (1:200)

2.pt 2) Cy 3-conjugated Streptavidin

- Jackson ImmunoResearch Lab: Code No. 016-160-084

-Dilution: (1:900)