

Jul 12, 2019

Version 1

Pig- Heart Neuonal and Fiber Immunocytochemistry V.1

DOI

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

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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: July 12, 2019

Protocol Integer ID: 22977

Keywords: pig, heart

- 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)