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## Pig- Heart Neuonal and Fiber Immunocytochemistry V.1

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Works for me

[dx.doi.org/10.17504/protocols.io.2n9gdh6](https://doi.org/10.17504/protocols.io.2n9gdh6)



scott john ⚡

- 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)



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