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Expression of molecular markers in mouse and human stellate ganglia

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ABSTRACT

Protocol for immunohistochemistry

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PROTOCOL CITATION

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Apr 01, 2022

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Jul 06, 2022

PROTOCOL INTEGER ID

60202

MATERIALS TEXT

Equipement:

Fisherbrand™ Superfrost™ Plus
Microscope Slides
Microscope Slides

Fisherbrand 12-550-15 [↗](#)

For frozen sections, cytocentrifuge preparations,
and standard Papanicolaou smears

Frosted: ¾" SuperFrost Tab
Type: Microscope Slide
Width (Metric): 25 mm
Corner Style: Regular
Length (Metric): 75 mm
Material: Glass
Color: White

Fisherbrand™ Cover Glasses: Rectangles
Cover Glasses

Fisherbrand 12-544-EP [↗](#)

Material: Borosilicate Glass
Length (Metric): 50 mm
Dimensions (L x W): 50 x 24 mm
For Use With: Microscope, Histology, Cytology,
Hematology, Microbiology
Product Type: Coverglass No. 1.5
Shape: Rectangular
Width (Metric): 24 mm
Thickness (Metric): 0.16 to 0.19 mm

ImmEDGE Hydrophobic Barrier Pen
Pen

Vector Laboratories NC9545623 [↗](#)

Applications: Immunohistochemistry /
Immunocytochemistry, Immunofluorescence, In
situ hybridization

Reagents:

 16% Paraformaldehyde Aqueous Solution **Fisher**

Scientific Catalog #50-980-488

 Phosphate Buffered Saline **Fisher**

Scientific Catalog #BP3994

 VECTASHIELD® Hardset™ Antifade Mounting Medium **Vector**

Laboratories Catalog #H-1400

 Triton X-100 **Sigma**

Aldrich Catalog #T8787-50ML

 Andwin Scientific Tissue-Tek™ CRYO-OCT Compound **Fisher**

Scientific Catalog #14-373-65

 DAPI **Fisher**

Scientific Catalog #D3571

 Normal Donkey Serum **Jackson**

Immunoresearch Catalog #017-000-121

Primary Antibodies:

A	B	C	D	E
Reagent	Dilution	Provider	Cat. No/SKU	RRID
Anti-Tyrosine Hydroxylase Antibody	1:100	Millipore-Sigma	AB152	AB_390204
Anti-Tyrosine Hydroxylase Antibody	1:400	Abcam	ab76442	AB_1524535
Anti-Neuropeptide Y Antibody	1:500	Sigma-Aldrich	M9528	AB_260814
Anti-Vasoactive Intestinal Peptide Antibody	1:500	Immunostar	20077	AB_572270

Secondary Antibodies:

A	B	C	D	E
Reagent	Dilution	Provider	Cat. No/SKU	RRID
Anti-Rabbit Secondary Antibody	1:500	ThermoFisher Scientific	A11034	AB_2576217
Anti-Rabbit Secondary Antibody	1:500	ThermoFisher Scientific	A31572	AB_162543
Anti-Chicken Secondary Antibody	1:500	ThermoFisher Scientific	A21449	AB_1500594

BEFORE STARTING

The human stellate ganglia (SG) were provided by Dr. Olujimi Ajijola at UCLA. The tissue was collected post-mortem from a 44-year-old female donor and dissected immediately after the donor's death.

The dissection procedure of murine SG is described in the "Tissue Harvesting" section of our protocol, "Injection of cholera toxin subunit B in the interscapular brown adipose fat and Stellate Ganglion dissection."



Injection of cholera toxin subunit B in the interscapular brown adipose fat and Stellate Ganglion dissection
by **Daniele Neri**

PREVIEW

RUN

Postfixation of the tissue

- Both mouse and human stellate ganglia (SG) were fixed in 4% PFA in 0.1M PB overnight at 4°C, washed with Phosphate Buffer Saline (PBS) 4°C for at least 1 h

Encasing and slicing

- Ganglia were encased in Optimal Cutting Temperature compound (Tissue-Tek) cut into 10 µm cryo-sections on slides, and stored at -80°C

Immunohistochemistry

- Thaw the sections at room temperature for at least 1 hour

- 4 Wash in PBS for 30 min twice at room temperature
- 5 Incubate in blocking buffer (5% donkey serum in PBS with 0.1% Triton X-100) for 1 hour at room temperature
- 6 Incubate the sections with primary antibodies diluted in blocking buffer overnight at 4C
- 7 Wash in PBS for 30 min three times at room temperature
- 8 Incubate the sections with secondary antibodies and DAPI for 1 hour at room temperature
- 9 Wash in PBS for 30 min three times at room temperature
- 10 Mount your slides using relevant mounting media and cover slip

Imaging

- 11 Image the sections using a confocal microscope

Analysis

- 12 Analyze the picture using ImageJ/FIJI