

# Combining Multiplex Fluorescence *in situ* Hybridization with Fluorescent Immunohistochemistry on Fresh Frozen or Fixed Mouse Brain Sections

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## URL

jove.com/video/61709

## Materials

Name	Company	Catalog Number	Comments
<b>ANIMALS</b>			
C57BL/6 mouse	Australian BioResources, Moss Vale	MGI: 2159769	
Phox2b-eGFP mouse	Australian BioResources, Moss Vale	MGI: 5776545	
<b>REAGENTS</b>			
Cyanoacrylate	Loctite		
Ethylene Glycol	Sigma-Aldrich	324558	
Heparin-Sodium	Clifford Hallam Healthcare	1070760	Consult local veterinary supplier or pharmacy.
Lethobarb (Sodium Pentobarbitol) Euthanasia Injection	Virbac (Australia) Pty Ltd	N/A	Consult a veterinarian for local pharmaceutical regulations regarding Sodium Pentobarbitol
Molecular grade agarose powder	Sigma Aldrich	5077	
OCT Compound, 118mL	Scigen Ltd	4586	
Paraformaldehyde, prilled, 95%	Sigma-Aldrich	441244-1KG	
Polyvinylpyrrolidone, average mol wt 40,000 (PVP-40)	Sigma-Aldrich	PVP40	
ProLong Gold Antifade Mountant	Invitrogen	P36930	With or without DAPI
RNAscope Multiplex Fluorescent Reagent Kit (up to 3-plex capability)	Advanced Cell Diagnostics, Inc. (ACD Bio)	ADV320850	Includes 50x Wash buffer and Protease III
RNase Away	Thermo-Fisher Scientific	7003	
Tris(hydroxymethyl)aminomethane	Sigma-Aldrich	252859	
Tween-20, for molecular biology	Sigma-Aldrich	P9416	
<b>EQUIPMENT</b>			
Benchtop incubator	Thermoline scientific micro incubator	Model: TEI-13G	
Brain Matrix, Mouse, 30g Adult, Coronal, 1mm	Ted Pella	15050	
Cryostat	Leica	CM1950	
Drawing-up needle (23 inch gauge)	BD	0288U07	

Hydrophobic Barrier Pen	Vector labs	H-4000	
Kimtech Science Kimwipes Delicate Task Wipes	Kimberley Clark Professional	34120	
Olympus BX51	Olympus	BX-51	
Peristaltic pump	Coleparmer Masterflex	L/S Series	
Retiga 2000R Digital Camera	QImaging	RET-2000R-F-CLR	colour camera
SuperFrost Plus Glass Slides (White)	Thermo-Fisher Scientific	4951PLUS4	
Vibrating Microtome (Vibratome)	Leica	VT1200S	
Whatman qualitative filter paper, Grade 1, 110 mm diameter	Merck	WHA1001110	
<b>SOFTWARES</b>			
CorelDRAW	Corel Corporation	Version 7	
FIJI (ImageJ Distribution)	Open Source/GNU General Public Licence (GPL)	N/A	ImageJ 2.x: Rueden, C. T.; Schindelin, J. & Hiner, M. C. et al. (2017), "ImageJ2: ImageJ for the next generation of scientific image data", BMC Bioinformatics 18:529, PMID 29187165, doi:10.1186/s12859-017-1934-z and Fiji: Schindelin, J.; Arganda-Carreras, I. & Frise, E. et al. (2012), "Fiji: an open-source platform for biological-image analysis", Nature methods 9(7): 676-682, PMID 22743772, doi:10.1038/nmeth.2019
<b>PRIMARY ANTIBODIES</b>			
Anti-Tyrosine Hydroxylase Antibody	Millipore Sigma	AB1542	Sheep polyclonal (1:1000 dilution), RRID: AB_90755
Anti-Tyrosine Hydroxylase Antibody, clone LNC1	Millipore Sigma	MAB318	Mouse monoclonal (1:1000 dilution), RRID: AB_2201528
Anti-Vesicular Acetylcholine Transporter (VAChT) Antibody	Sigma-Aldrich	ABN100	Goat polyclonal (1:1000 dilution), RRID: AB_2630394
GFP Antibody	Novus Biologicals	NB600-308	Rabbit polyclonal (1:1000 dilution), RRID: AB_10003058
Phox2b Antibody (B-11)	Santa Cruz Biotechnology	sc-376997	Mouse monoclonal (1:1000 dilution), RRID: AB_2813765
<b>SECONDARY ANTIBODIES</b>			
Alexa Fluor 488 AffiniPure Donkey Anti-Rabbit IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Ms, Rat, Shp Sr Prot)	Jackson ImmunoResearch	711-545-152	Donkey anti-Rabbit (1:400 dilution), RRID: AB_2313584
AMCA AffiniPure Donkey Anti-Sheep IgG (H+L) (min X Ck, GP, Sy Hms, Hrs, Hu, Ms, Rb, Rat Sr Prot)	Jackson ImmunoResearch	713-155-147	Donkey anti-Sheep (1:400 dilution), RRID: AB_AB_2340725
Cy5 AffiniPure Donkey Anti-Goat IgG (H+L) (min X Ck, GP, Sy Hms, Hrs, Hu, Ms, Rb, Rat Sr Prot)	Jackson ImmunoResearch	705-175-147	Donkey anti-Goat (1:400 dilution), RRID: AB_2340415
Cy5 AffiniPure Donkey Anti-Mouse IgG (H+L) (min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu, Rb, Rat, Shp Sr Prot)	Jackson ImmunoResearch	715-175-151	Donkey anti-Mouse (1:400 dilution), RRID: AB_2619678
Cy5 AffiniPure Donkey Anti-Sheep IgG (H+L) (min X Ck, GP, Sy Hms, Hrs, Hu, Ms, Rb, Rat Sr Prot)	Jackson ImmunoResearch	713-175-147	Donkey anti-Sheep (1:400 dilution), RRID: AB_2340730
<b>RNASCOPE PROBES</b>			
Galanin Receptor 1 oligonucleotide probe	ACDBio	448821-C1	targets bp 482 - 1669 (Genebank ref: NM_008082.2)
Glycine transporter 2 oligonucleotide probe	ACDBio	409741-C3	targets bp 925 - 2153 (Genebank ref: NM_148931.3)

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Phox2b oligonucleotide probe	ACDBio	407861-C2	targets bp 1617 - 2790 (Genebank ref: NM_008888.3)
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