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Immunofluorescence for pSer129 alpha-synuclein and total alpha-synuclein in mouse brain

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We use this protocol and it's working

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Abstract

This protocol details the immunofluorescence of brain section for phopho-serine129-alpha-synuclein and total alpha-synuclein

Materials

Buffers:

Sodium Citrate Buffer (500mL)

	A	B
	Sodium citrate	1.47g
	MQ H2O	500mL
	Adjust to 6.0 pH with 1N HCl	
	Tween-20	0.25 mL

store at 4^{o}C

10X Tris buffered saline (TBS) (1L)

	A	B
	Tris base	24.22g
	Sodium Chloride	87.66 g
	pH to 7.4 with hydrochloric acid	
	adjust to 1L with milliQ water	
	mix well. Autoclave or filter solution through 0.2µm filter into sterile bottle. Store at room temp.	

1X Tris buffered saline (500 mL)

	A	B
	10X TBS	50 mL
	milliQ water	450 mL
	Store at room temp	

Other reagents:

Recombinant Anti-Alpha-synuclein (phospho S129) antibody **Abcam Catalog #**ab51253

Anti-alpha beta Synuclein antibody [EP1646Y] **Abcam Catalog #**ab51252

Goat anti-Rabbit IgG (H L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488 **Invitrogen - Thermo Fisher Catalog #**A-11034

DAPI and Hoechst Nucleic Acid Stains **Invitrogen - Thermo Fisher Catalog #**H1399

Goat serum (Fisher NC9678224)

Thermo Scientific Chemicals Triton™ X-100, 98%, for molecular biology, DNAse, RNAse and Protease fre **Fisher Scientific Catalog #**AC327371000

Dilute to 10% w/v in TBS. Store at 4°C.

ProLong™ Gold Antifade Mountant **Invitrogen - Thermo Fisher Catalog #**P36934

Materials:

- Ice in ice bucket
- 6 well plates (non cell culture treated, Fisher 08-772-49; or the cheapest you can find).

-for rinses, blocking, and secondary antibody incubations 6 well plates can be re-used. After each use, clean with soapy water and scrub brush, rinse and let air dry.
-for primary antibody incubations, use new 6 well plate.

- Squirt bottles to easily add 1X TBS to wells.
- 15 mL Falcon tubes for blocking buffer, primary and secondary antibodies.

-for blocking, and secondary antibody incubations Falcon tubes can be re-used. After each use, clean with soapy water and scrub brush, rinse and let air dry.
-for primary antibodies, use new Falcon tube.

- Vortex
- Fisherbrand™ Superfrost™ Plus Microscope Slides **Fisher Scientific Catalog #**12-550-15
- 20, 200, 1000 µL pipetteman
- Glass hooks for transferring sections
- Fine paintbrush for mounting sections
- Orbital shaker

- netwell inserts (Fisher Scientific 07-200-213)

  Corning™ Costar™ Netwell™ Inserts **Fisher Scientific Catalog #**07-200-213

Troubleshooting

Day 1

2h 15m

1 Pre-warm sodium citrate buffer to 37 °C.

2 Place sections in netwell inserts (Fisher Scientific 07-200-213) in 6-well plate.

3 Wash 3X in cold 1X TBS for 5 min each with shaking (around 2 mL per well).

3.1 Wash in cold 1X TBS for 00:05:00 (1/3). 5m

3.2 Wash in cold 1X TBS for 00:05:00 (2/3). 5m

3.3 Wash in cold 1X TBS for 00:05:00 (3/3). 5m

4 Incubate at 37 °C for 01:00:00 in pre-heated sodium citrate buffer (about 2 mL/well). 1h

5 Wash 3X in 1X TBS for 5 min each.

5.1 Wash in 1X TBS for 00:05:00 (1/3). 5m

5.2 Wash in 1X TBS for 00:05:00 (2/3). 5m

5.3 Wash in 1X TBS for 00:05:00 (3/3). 5m

6 Block for 01:00:00 at 4 °C on shaker. Two mL of blocking buffer per well. Make sure sections are not folded on themselves. 1h

	A	B	C
	Blocking buffer		**10mL Block (for example)**
	5% Normal Goat Serum	→	500µL Normal Goat Serum
	0.1% Triton-X-100	→	100µL 10% Triton-X
	1X TBS	→	9.4mL 1X TBS

7 Place in primary antibody solution Overnight at 4 °C, shaking. For sections that cover an entire mouse forebbrain with 40 µL sections spaced 240 µL apart we use 2 mL per well. If you are just using a couple sections, you can use 0.5 mL per well in a new 24 well plate. Make sure sections are not folded on themselves. 1h

Note

- **Primary antibodies are diluted in 1X TBS and 5% goat serum**

**** NO MESH WELLS ****
****USE NEW PLATES FOR INCUBATION****

- Note catalog number and lot number (if available) in your notebook
- For pS129-α-synuclein (Abcam, ab51253) dilute 1:5000. Note that more concentrated antibody results in higher nonspecific background. It is possible to use at a more dilute concentration, but would need to be determined by user.
- For α+β synuclein (Abcam, ab51252) dilute 1:2500
- Can combine with other primary antibodies that were not generated in rabbit.

Day 2 15m

8 Wash 3X in cold 1X TBS for 5 min each with shaking.

8.1 Wash in cold 1X TBS for 00:05:00 with shaking (1/3). 5m

8.2 Wash in cold 1X TBS for 00:05:00 with shaking (2/3). 5m

8.3 Wash in cold 1X TBS for 00:05:00 with shaking (3/3). 5m

9 Place in secondary antibodies for 02:00:00 at 4 °C, shaking. Cover plate with foil to protect from light. 2h

Note

- **Secondary antibodies are diluted in 1X TBS and 5% goat serum**

**** NO MESH WELLS for incubation****

- Use goat anti-rabbit Alexa 488 1:500 (Thermofisher A-11034)
- Hoechst 33342 (Thermofisher H1399) also diluted 1:500

10 Wash 3X in 1X cold TBS for 5min each with shaking.

10.1 Wash in cold 1X TBS for 00:05:00 with shaking (1/3). 5m

10.2 Wash in cold 1X TBS for 00:05:00 with shaking (2/3). 5m

10.3 Wash in cold 1X TBS for 00:05:00 with shaking (3/3). 5m

11 Gently mount onto Superfrost plus slides in 1X TBS with a couple drops of 10% Tx-100.

Note

- Try your best not to rip sections or allow sections to fold.
- If you have sections representing entire brain, mount in order from rostral to caudal.
- Make sure the coverslip will cover all the sections. If you don't think it will cover the sections, use another slide. It's better to mount fewer sections and make sure the coverslip will cover them.

12 Allow to dry, coverslip using ProlongGold.

Note

- Pipette ProlongGold onto each section (about 30-50 µL).
- Slowly lower coverslip over sections to prevent bubbles.

Protocol references

Mahoney-CraneCL, Viswanathan M, Russell D, Curtiss RAC, Freire J, Bobba SS, Coyle SD, Kandebo M, Yao L, Wan B-L, Hatcher NG, Smith SM, Marcus JN, **Volpicelli-Daley LA.*** (2023) Neuronopathic GBA1L444P mutation accelerates glucosylsphingosine levels and formation of hippocampal alpha-synuclein inclusions. Journal of Neuroscience 43:501-421.