AUG 01, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.6qpvr36yovmk/v1

Protocol Citation: Tae-Un Han, Ellen Sidransky 2023. Immunohistochemistry (using floating section). **protocols.io** https://dx.doi.org/10.17504/protocols.io.6qpvr36yovmk/v1

Protocol status: Working
We use this protocol and it's working

Created: Jul 28, 2023

Immunohistochemistry (using floating section)

Tae-Un Han[1], Ellen Sidransky[1]

[1]National Institute of Health

Tae-Un Han

ABSTRACT

- This is an immunohistochemistry protocol used for evaluation of pathology in mouse brain.

- We successfully detected Gfap, Iba1, CD68, TH and alpha-Syn and phospho S129 alpha synuclein protein expression using this protocol.

- In this protocol, floating sections are mounted on slides first before starting blocking step. In this way, large number of sections can be stained more efficiently.

MATERIALS

30 um thickness of floating tissue sections in PBS with 0.1% sodium azide.

- Washing: Glass Coplin jar

- PBST: PBS 1X + 0.05% Tween20

- Normal Goat Serum Blocking Solution:
2% goat serum (blocking)
1%BSA (stabilizer)
0.1% cold fish skin gelatin (blocking)
0.1% Triton X-100 (penetration enhancer)
0.05% Tween 20 (detergent and surface tension reducer)
0.05% sodium azide (preservative)
0.01M PBS, pH 7.2
Mix well and store at 4 ºC for up to 6 months.

Last Modified: Aug 01, 2023

PROTOCOL integer ID: 85658

Keywords: ASAPCRN

- Primary Antibody Dilution Buffer
1%BSA (blocking & stabilizer)
0.1% cold fish skin gelatin (blocking)
0.5% Triton X-100 (penetration enhancer)
0.05% sodium azide (preservative)
0.01M PBS, pH 7.2-7.4
Mix well and store at 4 ºC. up to 6 months.

- Secondary Antibody Dilution Buffer:
0.01M PBS, pH 7.2
0.05% Tween 20

- List of antibodies used for neuropathology evaluation with IHC

	A	B	C	D
	Antibodies	Cat#	Company	dilution
	Anti-Synuclein α Antibody	AB5038	Sigma-Aldrich	1:1000
	Recombinant Anti-Alpha-synuclein (phospho S129) antibody [EP1536Y]	ab51253	Abcam	1:1000
	GFAP Antibody , Rabbit Polyclonal	16825-1-AP	Proteintech	1:4000
	Recombinant Anti-Iba1 antibody [EPR16588]	ab178846	Abcam	1:1000
	Anti-Tyrosine Hydroxylase	AB152	Sigma-Aldrich	1:1000
	Anti-CD68 antibody	ab125212	Abcam	1:1000
	Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) : 2nd antibody	ab150077	Abcam	1:1000

1 Mount floating sections onto slides and dry for 00:30:00 (<45 min), While section drying, PAP pen drawing line and dry 00:05:00. 35m

2 PBS rehydrate 00:10:00, Wash with PBS for 00:05:00 three times. 15m

3 Blocking sol 500 µL, incubate 01:00:00 at Room temperature. 1h

4 Replace buffer to primary antibody sol, incubate Overnight at 4 °C. 10m

5 Wash in PBST three times for 00:05:00, 00:10:00 and 00:05:00. 20m

6 Secondary antibody sol, incubate for 01:30:00 at Room temperature. 1h 30m

7 Wash in PBS three times for 00:05:00, 00:10:00 and 00:05:00. 20m

8 Mounting with Vectorshield mounting media