Preparation of the mouse brains for whole mount immunofluorescence as in Nam and Capecchi, 2020

Anesthetize the mouse

Transcardially perfuse the anesthetized mouse with PBS + heparin

Heparin
Sigma
H3393
Dissolve 100KU in 5 mL of PBS for a 20KU/mL solution. This is 1000x. Store @ 4 deg C.
Right before perfusions, dilute 1000-fold with PBS. Mix.

Perfuse PLP fixative with 2% formaldehyde (see page 4)

Dissect out the brain

Rinse away the excess fixative with PBS

Place the brain in a dish with PBS

Bisect the brain then dissect the two sides of the brain to reveal the ventricular wall

Post-fix in PLP fixative with 2% formaldehyde on nutator overnight @ 4 deg C

Rinse the brain with 1-2 changes of PBS

Block with 0.3 M glycine in PBS, pH 7.4 (see page 5) on nutator overnight @ 4 deg C

Store the brain in the glycine in PBS buffer @ 4 deg C

When ready to do the staining, transfer to a dish with PBS, dissect away the excess brain tissue

Permeabilize the brain with Triton X-100 in PBS @ room temperature

Immunostain as described in Nam and Capecchi, 2020 (see page 7)

The original inspiration for my PLP fixative, obtained from the Internet
I do something slightly different, see page 4

PLP-Fixative (a.k.a. "Nakane's"):

McLean & Nakane (1974)

Use: L-Lysine monohydrochloride Sigma L-5626 MW=182.6
Sodium (meta)periodate $INaO_4$ (also: $NaIO_4$) Sigma S-1878 MW=213.9
Na_2HPO_4
NaH_2PO_4

Final concentrations:
3% PFA
75 mM L-Lysine
10 mM $INaO_4$
0.1 M phosphate buffer

To make 100 mL fresh on day of use:

50 mL 0.2 M phosphate buffer pH 7.4 (from stock A+B)
32 mL distilled water
18 mL of 16% PFA
1.369 g L- Lysine
0.214 g $INaO_4$

Check pH; shouldn't be too far from 7.4

adapted from:

McLean IW, Nakane PK.
Periodate-lysine-paraformaldehyde fixative. A new fixation for immunoelectron microscopy.
J Histochem Cytochem 1974; 22:1077-108

0.2M Phosphate Buffer-4 Liters (pH 7.4):
17.66g Sodium Phosphate Monobasic 90.03g Sodium Phosphate Dibasic Heptahydrate 4 Liters ddH2O pH should be 7.4, if not adjust with 1.0N NaOH or 1.0N HCl
(3 Liters-13.25g Mono and 67.52g Dibasic)

2L 8.83 g 45.01 g

0.1 M sodium phosphate buffer (pH 7.4)

Add 3.1 g of $NaH_2PO_4 \cdot H_2O$ and 10.9 g of Na_2HPO_4 (anhydrous) to distilled H_2O to make a volume of 1 L. The pH of the final solution will be 7.4. This buffer can be stored for up to 1 mo at 4°C.

Here is what I do to make the fixative in Nam and Capecchi, 2020

Make 2x PB buffer with lysine and sodium metaperiodate, cool on ice
Make 2x formaldehyde in water, cool on ice

Right before the perfusions, I mix the two, then proceed as described on page 1

To make 100 mL of 2x PB buffer with lysine and periodate
Dissolve
2.738 g of lysine (Sigma catalog # L5626)
0.428 g of periodate (Sigma catalog # 71859)
in 100 mL 0.2 M (2x) PB, cool on ice

To make 200 mL of 2x PB Buffer with lysine and periodate
Dissolve
5.476 g of lysine
0.856 g of periodate
in 200 mL 0.2 M (2x) PB, cool on ice

To make 2 L of 0.2 M (2x) PB
Dissolve
8.83 g of sodium phosphate monobasic
45.01 g of sodium phosphate dibasic heptahydrate
in some purified water
Adjust pH to 7.4 with NaOH, add more water to volume, filter, store at room temperature

To make 4 L of 0.2 M (2x) PB
Dissolve
17.66 g of sodium phosphate monobasic
90.03 g of sodium phosphate dibasic heptahydrate
in some purified water
Adjust pH to 7.4 with NaOH, add more water to volume, filter, store at room temperature

To make 100 mL of 2x formaldehyde (4%)
Dissolve
4 g of paraformadehyde
in some purified water
Heat, add NaOH, wait to dissolve, add more water to volume, cool on ice

To make 200 mL of 2x formaldehyde (4%)
Dissolve
8 g of paraformadehyde
in some purified water
Heat, add NaOH, wait to dissolve, add more water to volume, cool on ice

After the post-fix, I block the brains with glycine in PBS buffer

0.3 M glycine in PBS, pH 7.4.

For 2 L, 45 g glycine.

Dissolve glycine in 1/10 volume of 10x PBS and some purified water, pH to 7.4 with NaOH, bring to volume with more water. Filter. Store @ 4 deg C.

Glycine
Sigma
ReagentPlus®, ≥99% (HPLC)
G7126

Permeabilization

Stock	**Final**	10000	20000	30000	40000	50000
20% (v/v) TX100	0.5% TX-100	250	500	750	1000	1250
	PBS	9750	19500	29250	39000	48750

Block

Stock	**Final**	500	1000	2000	3000	4000	5000	10000
100% (v/v) NGS	10% NGS	50	100	200	300	400	500	1000
20% (v/v) TX100	0.1% TX-100	2.5	5	10	15	20	25	50
2% (w/v) BSA	2% BSA in PBS	447.5	895	1790	2685	3580	4475	8950

Antibody

Stock	**Final**	500	1000	2000	3000	4000	5000	10000
100% (v/v) NGS	1% NGS	5	10	20	30	40	50	100
20% (v/v) TX100	0.1% TX-100	2.5	5	10	15	20	25	50
2% (w/v) BSA	0.5% BSA	125	250	500	750	1000	1250	2500
	PBS	367.5	735	1470	2205	2940	3675	7350

New Block

Stock	**Final**	500	1000	2000	3000	4000	5000	10000
100% (v/v) NGS	10% NGS	50	100	200	300	400	500	1000
20% (v/v) TX100	0.1% TX-100	2.5	5	10	15	20	25	50
2% (w/v) BSA	0.5% BSA in PBS	125	250	500	750	1000	1250	2500
	1:50 goat anti-ms Fab	10	20	40	60	80	100	200
	PBS	312.5	625	1250	1875	2500	3125	6250

Stocks

20% (v/v) TX-100 in water

Nam and Capecchi, 2020 immunofluorescence buffers

Block buffer								
Stock	**Final**	500	1000	2000	3000	4000	5000	10000
100% (v/v) NGS	10% NGS	50	100	200	300	400	500	1000
20% (v/v) TX-100	0.1% TX-100	2.5	5	10	15	20	25	50
2% (w/v) BSA in PBS	0.5% BSA in PBS	125	250	500	750	1000	1250	2500
1 mg/ml goat anti-ms Fab	20 ug/ml goat anti-ms Fab	10	20	40	60	80	100	200
	PBS	312.5	625	1250	1875	2500	3125	6250
Antibody buffer								
Stock	**Final**	500	1000	2000	3000	4000	5000	10000
100% (v/v) NGS	1% NGS	5	10	20	30	40	50	100
20% (v/v) TX-100	0.1% TX-100	2.5	5	10	15	20	25	50
2% (w/v) BSA in PBS	0.5% BSA in PBS	125	250	500	750	1000	1250	2500
	PBS	367.5	735	1470	2205	2940	3675	7350
Can also use PBST (0.1% TX-100)								
Normal Goat Serum from Vector Labs								
BSA from Jackson ImmunoResearch								
Goat anti-ms Fab from Jackson ImmunoResearch								

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