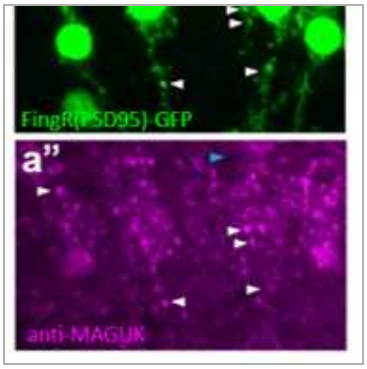

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Synaptic immunohistochemistry - wholemount via acetone permeabilization

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ABSTRACT

Whole-mount Immunohistochemistry – acetone permeabilization

Works with anti-MAGUK ab.

Modified from M Westerfield protocol.
Lavinia Sheets: T. Nicolson Lab September, 2010
Revised August 2011
Anya Suppermpool Synaptic (MAGUK) Version 2018

Anya's Benchling Protocol Archive at:
https://benchling.com/s/prt-nLJhuyARGTPcTi887wL1?m=slm-FGIOfoYTo8bWHpVnUZ0v

MATERIALS

PO_4buffer

8 parts0.1MNaH_2PO_4

2 parts0.1MNa_2HPO_4

BT buffer (make fresh)

1.0gsucrose

18.75µL0.2M$CaCl_2$

PO_4buffer to15mL

BT fix (make fresh)

1.2mLPO_4buffer

4.8mLBT buffer

2mL16% PFA (used bought Thermofisher fix)

PBS/BSA/DMSO

50mLPBS

0.5gBSA

500µLDMSO

Reagents

1 PO_4buffer

8 parts0.1MNaH_2PO_4

2 parts0.1MNa_2HPO_4

2 BT buffer (make fresh)

1.0gsucrose

18.75µL0.2M$CaCl_2$

PO_4buffer to15mL

3 BT fix (make fresh)

1.2mLPO_4buffer

4.8mLBT buffer

2mL16% PFA (used bought Thermofisher fix)

4 PBS/BSA/DMSO

50mLPBS

0.5gBSA

500µLDMSO

Day 1

5 Tricaine, Dechorionated beforehand (2dpf)

6 Fix 1.5-2h in BT fix (*use bought fix) at 4C

6.1 (less fix time higher SNR but mushy) 5h fixation works for 5dpf – 9dpf larvae, time may need to be adjusted for different ages

7 Replace fixative with PO4 buffer.
(Optional) Store at 4C overnight (shaking not necessary)

8 Wash for 5’ RT with shaking if continuing with permeabilization and blocking in same day

9 Chill a small volume (~50mL glass bottle) acetone at -20C at least 20’ prior to perm steps.
(optional) if rushed/forgotten, put acetone in -80C for a few minutes, but this could lead to over-permeabilization

10 Transfer larvae to glass vials using glass Pasteur pipette.
Larvae will be sticky so be careful while transferring between tubes!

11 Wash with dH2O 5’ RT with rocking.
Timing is critical for permeabilization steps—process tubes in same order for each step and adjust volume of solutions to allow for quick handling

12 Wash with cold acetone 5’ at -20C (put in freezer)

13 Wash with dH2O 5’ RT with rocking.
This step can go slightly longer (~10’) if necessary

14 Wash with PO4, 5’ at RT (Lavinia’s original protocol extra step)

15 Block >2h RT in PBS/BSA/DMSO + 2% goat serum.
(optional) can go overnight at 4C

16 Incubate in primary antibodies in PBS/BSA/DMSO 4C overnight.
Use ~1mL antibody mix per tube

Day 2

17 Remove primary antibody mix.
Add 1:1000 20%$NaN_3$to mix if saving for future use

18 Wash 5X+ withPBS/BSA/DMSO >20’ RT with rocking.
(optional) any washes after antibody incubation can go overnight at 4C if necessary

19 Incubate with secondary antibodies inPBS/BSA/DMSO 2-3h at RT or overnight at 4C in the dark

Day 3

20 Wash 3X withPBS/BSA/DMSO >20’ RT with rocking
(Wash withPBS/BSA/DMSO + DAPI (1:2000 dilution) >20’ RT with rocking)

21 Wash withPBS>20’ RT with rocking like 5x

22 Move to glycerol – 20%,40%,60%,80%

23 Mount and image using glycerol lens (did 20x before)

24

	Reagents	B	C
	Anti-MAGUK	1:500	MABN72 Anti-pan-MAGUK, clone K28/86
	Fix	#28906	ThermoFisher (from Ana Faro)
	tRFP	1:500	Anti-tRFP Rabbit Polyclonal (AB233 - evrogen)