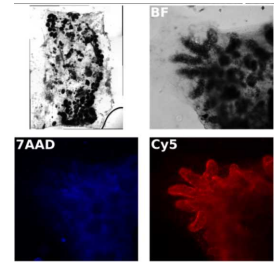


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Whole mount immunohistochemistry (WM-IHC)

DOI

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol has been successfully used with colonial tunicates has been adapted to these animals based on the following publication:

The onset of whole-body regeneration in Botryllus schlosseri: morphological and molecular characterization.



Materials

4% PFA

PBST (PBS + 0.1% Triton X-100)

PBST-SC (PBST + 3.9mM Sodium citrate)

PBST-GS (PBST + 5% Goat Serum)

primary and secondary antibodies

mounting medium

PAP pen

10x PBS, pH 7.2 (store for up to 2 months at RT)

	A	B	C
	Na ₂ HPO ₄ *2 H ₂ O	0.1M	17.8g
	KH ₂ PO ₄	18mM	2.4g
	NaCl	1.4M	80g
	KCl	27mM	2g
	water		1000mL

10x PBS (amounts calculated for 1L)

	A	B	C
	1x PBS		50mL
	Triton X-100	0.1%	50uL
	Sodium citrate	3.9mM	50mg

PBST-SC (amounts calculated for 50mL)



Fixation

1h 30m

- 1 Clean the slide with the colony of your interest.
Cleaning colonial ascidians.
- 2 Anesthetize the animals following this protocol. See Whole colony fixation V.1.
- 3 Fix the animals in 4% PFA  Overnight at  Room temperature . See Whole colony fixation V.1.
- 4 Wash twice in 3.3x PBS for  00:30:00 . 30m
- 5 Rinse quickly 3 times with 1x PBS and then leave in 1x PBS for  01:00:00 . 1h

Permeabilization and quenching

1h

- 6 Place samples in PBST-SC.
- 7 Shake  Overnight at  Room temperature .
- 8 Rinse once with 1x PBS.

Blocking and staining

3d 21h

- 9 Place samples in PBST-GS.



- 10 Shake at Room temperature for 02:00:00 . 2h
- 11 Stain your samples with primary antibody for 30:00:00 . 1d 6h
- 11.1 Dilute the primary antibody in PBST-GS according to the manufacturer.
- 11.2 On the slide, circle the system of interest with a PAP pen and wait 00:02:00 for the circle to dry. 2m
- 11.3 Place the slide flat into the humidity chamber.
- 11.4 Pipette the primary antibody solution on the circled system and incubate at 4 °C for 30:00:00 . 1d 6h
- 12 Wash four times in 1x PBST at Room temperature for 00:30:00 slowly on a linear. 30m
- 13 1d 6h
Stain your samples with secondary antibody for 30:00:00 .
- 13.1 Dilute the antibody in PBST-GS according to the manufacturer (keep solution and sample protected from the light!).
- 13.2 Incubate Overnight in darkness at 4 °C in the humidity chamber.
- 14 Wash four times in 1x PBST at Room temperature for 00:30:00 slowly on a linear shaker. 30m
- 15 Dry as much as possible.
- 16 Circle the sample with vaseline to build a custom-made chamber.



17 Mount with mounting medium, cover with a coverslip.

18 Image your slides directly or store them at  4 °C .