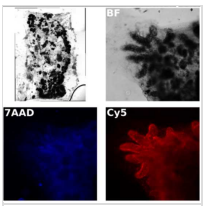

Jan 28, 2022

Whole mount immunohistochemistry (WM-IHC)

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dx.doi.org/10.17504/protocols.io.b2xfqfjn

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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.

DOI

dx.doi.org/10.17504/protocols.io.b2xfqfjn

Marta Wawrzyniak, Nathalie Weber, Simon Blanchoud 2022. Whole mount immunohistochemistry (WM-IHC). **protocols.io** https://dx.doi.org/10.17504/protocols.io.b2xfqfjn

colonial tunicates, WM-IHC, immunohistochemistry, ascidians

protocol ,

Dec 16, 2021

Jan 28, 2022

56007

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 antibobies
mounting medium
PAP pen
10x PBS, pH 7.2 (store for up to 2 moths at RT)

A	B	C
Na2HPO4*2H2O	0.1M	17.8g
KH2PO4	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

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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.

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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 Stain your samples with secondary antiboby for **30:00:00** . 1d 6h

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.

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17 Mount with mounting medium, cover with a coverslip.

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

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