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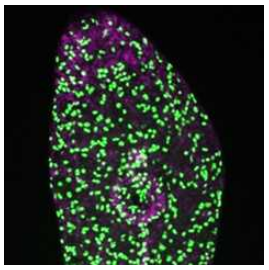
EdU Staining Protocol in juvenile F. hepatica



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

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LIVER FLUKE MOTOR FUNCTION AND PARASITE CONTROL: EXPLOITING A 'TARGET VALIDATION TOOLBOX' AS A DRUG SCREEN-INTERFACE FOR FLUKICIDE DISCOVERY
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Abstract

This protocol describes the process of labelling and whole mount staining proliferative cells in *in vitro* juvenile *Fasciola hepatica* with the thymidine analogue 5-ethynyl-2′-deoxyuridine (EdU). The protocol is based on the *Schistosoma mansoni* EdU staining protocol developed by the Collins and Newmark groups. A full copy of that protocol is available here - http://collinslab.org/PDF/Whole_mount_EdU_Smansoni.pdf.

Guidelines

Fixation

While flat fixing is necessary for worms grown for more than three weeks *in vitro* younger worms may be suitable for free fixing in 4%FA.

Materials

CS50 - 50% Chicken Serum, New Zealand Origin **Thermo Fisher Catalog #**16110082 and 50% RPMI 1640 Medium, no phenol red **Thermo Fisher Catalog #**11835105 with x1 Antibiotic Antimycotic Solution (100×), Stabilized **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A5955

PBSTx - 1X PBS + 0.3% Triton X-100 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T8787-50ML

4%FA - 500 µL Formaldehyde solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**F8775-25ML with 4 mL PBSTx

4%PFA - Add 8 g Paraformaldehyde to 100 mL millipore water, stir/heat **in a flow hood** for 1 h between 55 °C and 60 °C on hotplate (**NB. must NOT go above 60^{o}C**; **if it does, start again**). Following 1 h heating turn off heat and add NaOH dropwise until solution goes clear before adding 100 mL 2X PBS. and mix. Store at 4 °C for 2 weeks or -20 °C in aliquots for 6 months.

EdU Detection Solution

	A	B	C	D	E
	Reagent	**200 (µl)**	**1 ml (µl)**	**5ml (ml)**	**10ml (ml)**
	PBS	158	789	3.945	7.89
	100mM Copper Protectant in Water (Store 4oC)	2	10	0.05	0.1
	10mM AlexaFluor (488 or 545)/6-FAM azide	0.2	1	0.005	0.01
	500mM Ascorbic Acid (Make fresh – 88 mg in 1 ml PBS)	40	200	1	2

Copper protectant - Copper(II) sulfate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**451657

Alexafluor 488 - Azide-fluor 488 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**760765

6-FAM azide - 6-Fam Azide **Metabion**

DAPI solution - 1:1000 of [M] 1 mg/mL stock in PBSTx

Protocol materials

Triton X-100 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T8787-50ML

RPMI 1640 Medium, no phenol red **Thermo Fisher Catalog #**11835105

Azide-fluor 488 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**760765

Copper(II) sulfate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**451657

Chicken Serum, New Zealand Origin **Thermo Fisher Catalog #**16110082

6-Fam Azide **Metabion**

Antibiotic Antimycotic Solution (100×), Stabilized **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A5955

Formaldehyde solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**F8775-25ML

VECTASHIELD Mounting Medium **Vector Laboratories Catalog #**H-1000

Safety warnings

EdU Mutagen
As a thymidine analogue EdU is known for germ cell mutagenicity and reproductive toxicity. Particular care should be taken when disposing of the chemical and around pregnant mothers.

In vitro addition of EdU to juvenile *F. hepatica*

1d

1 Juvenile liver fluke are cultured in 50% Chicken serum (CS50) as described in McCusker et al. (2016).

2 10 µL of [M] 10 millimolar (mM) 5-Ethynyl-2'-deoxyuridine (EdU) is mixed with 190 µL pre-warmed CS50 and added to worms for between 6 and 24 h.

STEP CASE

Stain immediately following EdU incubation

15 steps

Fixation

3 Add 20 µL **4% FA/4% PFA** to a petri dish.

4 Pipette worms in into **4% FA/4% PFA** and immediately flatten with coverslip and weight (e.g. half full 15 mL falcon tube) for 10 min.

5 Add **4% FA/4% PFA** to float coverslip. Lift coverslip and move worms into 1.5 mL eppendorf with 1 mL **4% FA/4% PFA**. Place tube on a rotator for further 10 min (4%FA)/2 h (4%PFA) at Room temperature **OR** O/N at 4 °C.

OPTIONAL Storage Step

6 Wash in 1 mL **PBSTx** for 10 min.

7 Wash in 1 mL 1:1 **PBSTx**:Methanol for 10 min.

8 Store in 1 mL methanol at -20 °C until required.

9 Rehydrate in 1 mL 50:50 **PBSTx**:Methanol for 10 min.

Staining

10 x2 washes in 1 mL **PBSTx** for 10 min each.

11 Incubate in 1 mL fresh **Proteinase K Solution** for 15 min at Room temperature .

12 Postfix in 1 mL **4%FA** for 10 min.

13 Wash in 1 mL **PBSTx** for 10 min.

14 Incubate in **EdU Detection Solution** for 30 min at Room temperature . **NB: cover with tinfoil from this point on.**

15 x2 washes in 1 mL **PBSTx** for 5 min each

16 Incubate in 1 mL **DAPI Solution** for 20 min at Room temperature or O/N at 4 °C .

17 Rinse in **PBSTx** and mount in VECTASHIELD Mounting Medium **Vector Laboratories Catalog #**H-1000 .

Protocol references

McCusker, P., McVeigh, P., Rathinasamy, V., Toet, H., McCammick, E., O'Connor, A., Marks, N. J., Mousley, A., Brennan, G. P., Halton, D. W., Spithill, T. W., & Maule, A. G. (2016). Stimulating Neoblast-Like Cell Proliferation in Juvenile Fasciola hepatica Supports Growth and Progression towards the Adult Phenotype In Vitro. PLoS Neglected Tropical Diseases, 10(9), [e0004994]. https://doi.org/10.1371/journal.pntd.0004994