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Wholemount Edu Staining (Zebrafish Larvae)

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Protocol status: Working
We use this protocol and it's working for larvae between 1-5 dpf. Our analysis is often focused on the skin tissue, but we saw cells in other part of the fish were also stained.

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Keywords: zebrafish embryo, evaluating cell proliferation, tissue cultured cell, assays by invitrogen, cultured cell, zebrafish larvae, cell biology, cell proliferation in vivo, wholemount edu staining, cell proliferation, cell, crucial edu injection step, assay, cancer biology, staining protocol, variable edu incorporation efficiency, inconsistent results due to variable edu incorporation efficiency, developmental biology, field of developmental biology, fast staining speed, vivo, standard kit from invitrogen

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Disclaimer

Abstract

Methods for evaluating cell proliferation are important for research in the field of developmental biology, cancer biology and cell biology. Recent years, Click-iT EdU Assays by Invitrogen is gaining popularity due to its fast staining speed, mild tissue treatment condition, reproducibility and specificity. However, the standard kit from Invitrogen is designed for tissue cultured cells, hence the manufactory provided protocol is optimized for cells cultured on coverslips. EdU staining became a popular method to evaluate cell proliferation in vivo in zebrafish embryos. However, the protocols that we have tested in the past often yield inconsistent results due to variable EdU incorporation efficiency. Here we describe an EdU staining protocol that we optimized for zebrafish larvae, which incorporated a crucial EdU injection step. Our protocol was used successfully by many new students in training, without any issues.

Troubleshooting

EdU staining

4h 50m

1 Inject 2nl bolus of 500uM EdU into the yolk of embryos — 30m

> Note
>
> Reagents for EdU staining can be purchased from - https://www.thermofisher.com/uk/en/home/references/protocols/cell-and-tissue-analysis/protocols/click-it-plus-edu-imaging-protocol.html
>
> embryos were immobilized in 3% methylcellulose in 0.3 x DANEAU's solution (or E3) and returned to induction solution after injection.

2 Incubate at **28.5°C** in the dark for **2.5 hours (this can be between 2 -3 hours, but be consistent between experiments; we have done 2 hours)** — 2h 30m

3 Fix in 4% PFA (**30min**, Room temperature) 25 °C 00:30:00 — 30m

4 Wash in 0.1% PBT (**5min**, RT shaking) 30 rpm, 25°C, 00:05:00 repeat 3 times — 15m

5 Wash in 3% BSA, Dilute in PBT (**5min**, RT shaking) -grams/100ml, i.e. 3g/100ml, 300mg in 10ml. 30 rpm, 25°C — 5m

6 Block in 3% BSA, Dilute in PBT (**1h**, shaking) 30 rpm, 25°C, 01:00:00 — 1h

7 Incubate in staining solution (as per kit protocol) – we use 250ul per sample (10-15 larvae) **30 min**, 30 rpm, 25°C, 00:30:00 250 µL per 10-15 larvae — 30m

	1	2	4	5	10	25	50
1X Click-iT® reaction buffer	440 µL	880 µL	1.84 mL	2.25 mL	4.4 mL	10.9 mL	21.9 mL
Copper protectant	10 µL	20 µL	40 µL	50 µL	100 µL	250 µL	500 µL
Alexa Fluor® picolyl azide (Component B)	1.2 µL	2.5 µL	5 µL	6 µL	12.5 µL	31 µL	62 µL
1X Click-iT® EdU buffer additive	50 µL	100 µL	200 µL	250 µL	500 µL	1.25 mL	2.5 mL
Total volume	500 µL	1 mL	2 mL	2.5 mL	5 mL	12.5 mL	25 mL

*Note: Add the ingredients in the order listed in the table.

Immunostaining in dark (EGFP or hRAS)

1d 17h 50m

8 Wash in PBT (**5min**, RT shaking **x3)** 30 rpm, 25°C, 00:05:00 X3 — 15m

9 Wash in 5% Goat Serum, Dilute in PBT (**5min**, RT shaking) 30 rpm, 25°C, 00:05:00 — 5m

10 Block in 5% Goat Serum, Dilute in PBT (**at least 2h**, RT shaking) — 2h
30 rpm, 25°C, 02:00:00

11 Incubate with anti-GFP Primary Ab 1:200, dilute in 5% Goat Serum in PBT (O/N, +4 °C shaking) — 16h
30 rpm, 4°C, 16:00:00 Over night
250 µL 10-30 larvae

Note

GFP (D5.1)XP Rabbit mAB (Cell Signalling Technology 2956)

12 Wash in PBT (**25-30 min**, RT shaking) **x 8-10 à >4 hours in total!!!** 5h
30 rpm, 25°C, 00:30:00 X10

13 Incubate with Secondary Ab [] 1:250, Dilute in 3% Goat Serum in PBT (overnight at +4 °C -- prefered) 16h
30 rpm, 4°C, 16:00:00 Or 25C 2 hours

> Note
>
> Alexa Fluor 488 goat anti-rabbit IgG (H+L) (Invitrogen A11008)]
> This step over night is preferred, however, 2 hours at RT also works.

14 Wash in PBT (**15 min**, RT shaking) **x4** 1h
30 rpm, 25°C, 00:15:00 X 4

15 Stain with Hoechst 33258 Dilute 1 in 1000 in PBT (**30mins**, RT shaking) 30m
30 rpm, 25°C, 00:30:00 in dark

> Note
>
> Or other nuclear stain such as DAPI

16 Wash in PBT (15mins, PBT) **x4** 1h
30 rpm, 25°C, 00:15:00 X 4

Embed and Image 10m

17 Incubate in AF1 solution. 10m

> Note
>
> https://www.cntech-labsupplies.co.uk/products/sample-preparation/film-forming-polymer-solutions
>
> Sample can be stored in the dark for few weeks

18 Mounting with cover-slip and glass slides.