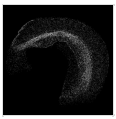

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Click histochemistry for whole-mount staining of brain structures

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1 Works for me dx.doi.org/10.17504/protocols.io.8rqhv5w

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ABSTRACT

Labeling of the replicating DNA with synthetic thymidine analogs is commonly used for marking the dividing cells. However, until now this method has only been applied to histological sections. A growing number of current approaches for three-dimensional visualization of large tissue samples requires detection of dividing cells within whole organs. Here we describe a method for labeling dividing cells with 5-ethynyl-2'-deoxyuridine (EdU) and their further detection in whole brain structures (for example, hippocampus) using the Cu (I) -catalyzed [3 + 2] cycloaddition reaction (so-called click-reaction). The presented method can be used for brain neurogenesis studies as well as for whole-mount staining of any preparations in which the terminal ethynyl group has been introduced.

• New click histochemistry method based on Cu (I) -catalyzed [3 + 2] cycloaddition reaction allows whole-mount staining of brain structures and other tissues.

• Our whole-mount click histochemistry method allows to visualize dividing cells in 3D and can be used in neurogenesis studies, i.e. for birthdating dividing early progenitors and further tracking of proliferation, survival, migration, differentiation, and fate of their progeny.

• Our whole-mount click histochemistry staining demonstrates high staining specificity, high signal intensity, and low background levels in young and adult mouse brain tissue.

EXTERNAL LINK

https://www.sciencedirect.com/science/article/pii/S2215016119302353

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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2019_Click histochemistry for whole-mount staining of brain structures.pdf

GUIDELINES

Microtubes must be filled fully to prevent the contact of specimens with air and development of autofluorescence on the samples' surface.

All procedures described below must be performed with constant stirring and at **Room temperature**, unless otherwise specified.

MATERIALS

NAME	CATALOG #	VENDOR
Paraformaldehyde	P6148	Sigma Aldrich
Alexa Fluor™ 555 Azide, Triethylammonium Salt	A20012	Thermo Fisher
5-ethynyl-2'-deoxyuridine	40540	Lumiprobe

NAME	CATALOG #	VENDOR
PBS Tablets	524650	Millipore Sigma
Chloral hydrate	C8383	Sigma Aldrich
DMSO	W387520	Sigma Aldrich
Methanol	34860	Sigma Aldrich
Sodium L-ascorbate	A4034	Sigma Aldrich
Copper(II) sulfate	61230	Sigma Aldrich
Dibenzyl ether	108014	Sigma Aldrich
Hydrogen peroxide 30%	8.22287	Sigma Aldrich
UltraPure 0.5M EDTA pH 8.0	15575020	Invitrogen - Thermo Fisher
Saponin	84510	Sigma Aldrich
Triton X-100	T8787	Sigma Aldrich
UltraPure™ 1M Tris-HCl pH 8.0	15568025	Thermo Fisher Scientific

MATERIALS TEXT

Step 1: tissue sample preparation

- 5-ethynyl-2'-deoxyuridine, 6.15 mg/ml
- 0.1 M phosphate buffer (PBS) pH 7.4
- 4% paraformaldehyde (PFA) in PBS, pH 7.4 at 4 °C
- 15% chloral hydrate
- 2 ml microtubes
- 1 ml syringes

Step 2: pretreatment and storage

- Eppendorf ThermoMixer Temperature Control Device (EppendorfTM 5382000023)
- DMSO
- 100% methanol
- 50% methanol
- 25% methanol
- 12.5% methanol
- 30% H_2O_2 stock solution
- 10% Triton X-100 stock solution in PBS
- 10% saponin stock solution in PBS
- 0.1 M Tris-HCl buffer (pH 8.0)

Step 3: click reaction

- DMSO
- 10% Triton X-100 stock solution in PBS
- 10% saponin stock solution in PBS
- 0.1 M Tris-HCl buffer (pH 8.0)
- 1 M sodium ascorbate stock solution (freshly prepared)
- 100 mM $CuSO_4$ stock solution
- 1 mM fluorescent azide (e.g. Alexa Fluor 555 Azide, Triethylammonium Salt, A20012, Invitrogen) stock solution in DMSO
- 0.5 M EDTA (pH 8.0) stock solution

Step 4: clearing

- 25% methanol
- 50% methanol
- 75% methanol
- 100% methanol
- Dibenzyl ether

SAFETY WARNINGS

Paraformaldehyde requires careful handling since it is highly toxic and potentially carcinogenic. Work only in a fume hood.

Methanol requires careful handling since it is highly toxic and highly inflammable liquid.

The methanol/DMSO solution should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues.

Dibenzyl ether requires careful handling since it is strong non-polar solvent. Use only polyethylene and glass labware.

BEFORE STARTING

A method described here allows staining whole brain structures using the Cu (I) -catalyzed [3 + 2] cycloaddition reaction (so-called click-reaction) between the terminal ethynyl group incorporated into DNA upon 5-ethynyl-2'-deoxyuridine (EdU) injection and fluorescently labeled azide. This method can be used in neurogenesis studies, i.e. for birthdating of dividing early progenitors and further tracking of proliferation, survival, migration, differentiation, and fate of their progeny. This whole-mount click method is based on the original protocol for staining tissue sections developed by Salic and Mitchison (2008). The following procedures were developed and optimized for whole hippocampus preparation staining and imaging.

Salic A, Mitchison TJ (2008). A chemical method for fast and sensitive detection of DNA synthesis in vivo.. Proceedings of the National Academy of Sciences of the United States of America. https://doi.org/10.1073/pnas.0712168105

All procedures described below must be performed with constant stirring and at **Room temperature**, unless otherwise specified.

Tissue sample preparation

1 Inject animal with a synthetic thymidine analogue 5-ethynyl-2'-deoxyuridine at a dose of 40-123 mg/kg. The time to euthanasia may vary depending on the specific research question.

2 Anesthetize animal with chloral hydrate (10 mg/kg) immediately before euthanasia.
00:10:00

3 Perfuse animal intracardially with 30 ml of PBS and 30 ml of cold 4% paraformaldehyde, pH 7.4.
01:15:00

Paraformaldehyde requires careful handling since it is highly toxic and potentially carcinogenic. Work only in a fume hood .

4 Dissect the brain in 1 h after perfusion.
00:05:00

5 Postfix the brain samples by overnight (ON) immersion in 4% PFA at 4 °C .

14:00:00

6

Rinse the brain twice with PBS for 2 h each time.

04:00:00

7

Remove the cerebral cortex from the fixed hemispheres using a microsurgical spatula, then isolate the hippocampi and place it in 2 ml microtube with cold PBS.

00:10:00

Pretreatment and storage

8 Incubate the specimen in Dent solution (100% methanol/DMSO, in the ratio of 4:1) for 2 h at 4 °C .

02:00:00

Methanol requires careful handling since it is highly toxic and highly inflammable liquid.

The methanol/DMSO solution should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues.

9 To destroy endogenous pigments, bleach the specimen in Dent bleach solution (100% methanol/DMSO/30% H_2O_2, in the ratio of 4:1:1) in bright light for 2 h until the samples are completely white.

02:00:00

10 After bleaching, rinse the specimen three times in 100% methanol for 1 h each time.

03:00:00

11

At this stage, specimens can be stored in 100% methanol at -70 °C . It is important to warm up the preparations gradually after storage to preserve the morphology. For this, transfer the sample first to -20 °C , then to 4 °C , and only after that brought it to Room temperature .

12 Rehydrate samples stepwise in 75%, 50%, 25% and PBS for 1 h in each solution.

04:00:00

13

Wash rehydrated sample twice in PBS for 1 h each time.

02:00:00

14 Permeabilize the preparation in 2% saponin and 5% DMSO solution in PBS for 1 h at 37 °C .

01:00:00

15 After permeabilization, wash the preparation in Tris-HCl buffer (pH 8.0) with 0.2% Triton X-100 for 1 h.

01:00:00

Click reaction

16 ⚠

Incubate the specimen in click reaction solution (Tris-HCl buffer containing 5% DMSO, 0.2% Triton X-100, 0.2% saponin, 100 mM sodium ascorbate, 1 mM $CuSO_4$ and 10 mM fluorescent azide) for 2 h in the dark.

02:00:00

Click reaction solution must be prepared immediately before staining. 1 M sodium ascorbate stock solution must be prepared from the powder immediately before the click reaction.

17 Stop the click reaction by three 1 h incubations in Tris-HCl buffer with 5% DMSO, 0.2% Triton X-100 and 0.1 M EDTA.

03:00:00

18 ⏸

Wash stained preparation twice for 1 h with PBS containing 5% DMSO and 0.2% Triton X-100 and twice with PBS for 1 h each time. Final rinsing with PBS was done overnight at 4 °C .

18:00:00

Clearing

19 ⏸

Dehydrate the preparation in 25%, 50%, and 75% methanol and three times in 100% methanol for 1 h for each incubation.

06:00:00

20 ⏸

Clear the preparation for ON in dibenzyl ether (DBE) in the dark at 4 °C . Samples can be stored in clearing solution.

14:00:00

Dibenzyl ether requires careful handling since it is strong non-polar solvent. Use only compatible plastic (polyethylene etc.) and glass labware.