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Thymidine analog labeling of proliferating cells

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We use this protocol and it's working

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Abstract

This protocol describes the procedure used by the Seifert Lab to label cycling cells with thymidine analogs to track cell proliferation in injured tissue. EdU is used to label cycling cells before the injury, and BrdU after, thereby assessing cell proliferation in response to injury. It has been successfully used in rodents (*Mus musculus* and *Acomys dimidiatus* - spiny mouse), zebrafish (*Danio rerio*) and axlotl (*Ambystoma mexicanum*).

Note that any protocol involving animals should be reviewed and approved by your Institutional Animal Care and Use Committee (IACUC) before use.

Materials

1. Thymidine analogs diluted in DMSO (EdU and BrdU)
2. Sterile saline solution (0.9% NaCl)
3. 1.5 mL Eppendorf tubes
4. Syringe (2 mL capacity, suitable gauge for injection)
5. Appropriate injection needles (e.g., 25-30 gauge for intraperitoneal injection)
6. Animals, e.g. *Acomys dimidiatus* (spiny mice), *Mus musculus*, *Ambystoma mexicanum* (axolotls), *Danio rerio* (zebrafish)

Protocol materials

BrdU (5-Bromo-2´-Deoxyuridine) **Thermo Fisher Catalog #**B23151

EdU (5-ethynyl-2’-deoxyuridine) **Thermo Fisher Catalog #**A10044

Troubleshooting

1 *For Acomys, Mus, or Ambystoma:*
Dilute EdU (5-ethynyl-2’-deoxyuridine) **Thermo Fisher Catalog #**A10044 from stock in sterile saline to a final concentration of [M] 10 µg/g body weight

2 *For Acomys, Mus, or Ambystoma:*
Dilute BrdU (5-Bromo-2´-Deoxyuridine) **Thermo Fisher Catalog #**B23151 from stock in sterile saline to a final concentration of [M] 1 µg/g body weight

3 *For Acomys, Mus, or Ambystoma:*
Transfer the thymidine analog solution to a 2 mL syringe appropriate for intraperitoneal injection (25-30 gauge)

4 *For Acomys, Mus, or Ambystoma:*
Inject EdU intraperitoneally three times at 3-hour intervals (i.e., 0h, 3h, and 6h)

5 *For Acomys, Mus, or Ambystoma:*
Collect tissue at 9h (for assessment of baseline cell proliferation)

6 *For Acomys, Mus, or Ambystoma:*
At desired time following injury, inject BrdU intraperitoneally three times at 3-hour intervals (i.e., 0h, 3h, and 6h)

7 *For Acomys, Mus, or Ambystoma:*
Collect tissue at 9h (for assessment of injury-induced cell proliferation)

8 *For Danio:*
Prepare a final solution of [M] 0.4 millimolar (mM) EdU or BrdU in zebrafish water

9 *For Danio:*
Place zebrafish in the prepared water for a 09:00:00 exposure period to allow analog uptake before collecting tissue