

abcam

BrdU staining protocol

A guide to BrdU staining

Introduction

BrdU labeling can be performed *in vitro* for cell lines and primary cell cultures, or *in vivo* for labeling cells within a living animal. BrdU is incorporated into replicating DNA and can be detected using anti-BrdU antibodies.

After BrdU labeling, an additional DNA hydrolysis step (sometimes referred to as a DNA denaturing step) may be required after fixation and permeabilization to allow the anti-BrdU antibody access to the BrdU within the DNA .

[BrdU antibodies](#) can be used in conjunction with cell type markers such as [Ki67](#), [doublecortin](#), and [NeuN](#) to identify proliferating cells and newly differentiated neurons.

BrdU labeling: in vitro labeling of cells

1. Prepare a 10 mM stock solution of [BrdU \(ab142567\)](#) by dissolving 3 mg of BrdU in 1 mL water.
2. Dilute the 10 mM BrdU stock solution in cell culture medium to make a 10 µM BrdU labeling solution.
3. Filter the 10 µM BrdU labeling solution through a 0.2 µm filter under sterile conditions.
4. Remove the existing culture medium from the cells and replace with 10 µM labeling solution.
5. Incubate the cells in the BrdU labeling solution for 1–24 hours at 37°C in a CO₂ incubator.

Note: BrdU incubation time depends on how rapidly the cells divide. Primary cells may need up to 24 hours, while rapidly proliferating cell lines may only need one hour. The exact time required to achieve the optimal signal-to-noise ratio should be optimized.

6. Remove the BrdU labeling solution from the cells and wash twice in PBS for about 5 seconds per wash.
7. Wash three more times with PBS for two minutes each.
8. Fix and permeabilize cells according to [standard immunocytochemistry \(ICC\) protocols](#). However, before proceeding with immunostaining, refer to the DNA hydrolysis step below.

BrdU labeling: in vivo labeling

There are several methods for labeling cells *in vivo* with BrdU. Two commonly used methods are intraperitoneal injection and oral administration.

Intraperitoneal injection

1. Dilute BrdU in PBS to make a sterile solution of 10 mg/mL.
2. For mice, as a general rule, inject the BrdU solution to a concentration of 100 mg/kg.
3. After treatment with BrdU, the animals can be sacrificed according to your lab's approved procedures.
4. Fix and process tissue according to [standard immunohistochemistry \(IHC\) protocols](#). However, before continuing with immunostaining, refer to the DNA hydrolysis step below.

Note: BrdU incorporation into rapidly dividing tissues, such as the small intestine, will be detectable as soon as 30 minutes post-injection. However, for most tissue you may need to wait up to 24 hours. The exact treatment time and dosage will need to be optimized for your tissue of interest.

Oral administration

Oral administration of BrdU is a non-invasive procedure and therefore useful for extended BrdU administration, although it may introduce variability into experiments due to lack of control over an animal's water consumption.

1. Dilute BrdU to 0.8 mg/mL in drinking water. Prepare this fresh and change daily.
2. After treatment with BrdU, the animals can then be sacrificed according to standard protocols.
3. Fix and process tissue according to [standard IHC protocols](#). However, before immunostaining refer to the DNA hydrolysis step below.

Note: for mice, 225 mg/kg per day of BrdU (calculated by measuring water consumption volumes per animal) should achieve sufficient BrdU labeling. However, the exact dose should be optimized for individual experimental conditions.

DNA hydrolysis

Preparation

Sodium borate buffer: 3.8g sodium borate (MW=381.4) + 100 ml distilled water. Adjust pH with NaOH.

Cells

1. Incubate cells in 1–2.5 M HCL for 10 minutes to 1 hour at room temperature. The exact HCl concentration and incubation time should be optimized for your experiment. If using a shorter incubation time, incubating at 37°C may be more effective than room temperature.
2. Optional step: remove the HCl and neutralize with 0.1 M sodium borate buffer pH 8.5 for 30 minutes at room temperature.
3. Wash three times in PBS.
4. Continue with immunostaining according to [standard immunocytochemistry \(ICC\) protocols](#).

Tissue sections

Note: if using paraffin-embedded sections, ensure they are de-waxed before proceeding. [Read our deparaffinization protocol here.](#)

1. Incubate sections in 1–2 M HCL for 30 minutes to 1 hour. The exact HCl concentration and incubation time should be optimized for your experiment. If using a shorter incubation time, incubating at 37°C may be more effective than room temperature.
2. Optional step: neutralize sections by incubating in 0.1 M sodium borate buffer pH 8.5 for 10 minutes at room temperature.
3. Wash three times in PBS for about 5 seconds per wash.
4. Continue with immunostaining according to [standard IHC protocols](#).

Note: some researchers have reported that they don't perform the HCl hydrolysis step and simply perform heat-induced epitope retrieval before continuing with immunostaining.

Co-staining with anti-BrdU

BrdU can be used in conjunction with other antibodies to identify proliferating and newly differentiated neurons. See below for our suggestions.

Ki67

A cellular marker for proliferation, the Ki67 protein is present in cells at cycle phases G1, S, G2 and M, but absent in resting (G0) cells. Ki67 antibodies can be used instead of, or in conjunction with, BrdU to label proliferating neurons.

[Read more about our knockout-validated Ki67 antibodies.](#)

Doublecortin

A microtubule-associated phosphoprotein expressed by immature neurons. Doublecortin antibodies can be used in conjunction with BrdU to identify immature post-mitotic neurons.

[Browse our doublecortin antibodies.](#)

NeuN

A marker of mature neurons that can be used in conjunction with BrdU staining to identify newly differentiated neurons.

[Read about the advantages of our NeuN RabMAb® antibody](#)

Want to keep up to date with the latest products and research tools from Abcam? [Sign up](#) to get your personalized recommendations.