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In Situ BrdU Cell Proliferation Assay Kit

Catalog Number: A055

Table 1. Kit Components and Storage

Material	Amount	Concentration	Storage	Stability
BrdU (Component A)	**50 mg**	**-**	**4 °C**	**The product is stable for at least six months when stored as directed.**
BrdU Detection Antibody (Component B)	**50 µL**	**100X**		
Anti-mouse Andy Fluor 488-labeled Antibody (Component C)	**50 µL**	**100X**		
Antibody Diluent (Component D)	**50 mL**	**1X**		

Number of assays: Sufficient material is supplied for 50 coverslips based on the protocol below.

Introduction

Detecting cell proliferation is a fundamental method for assessing cell health, determining genotoxicity, and evaluating anti-cancer drugs. The most accurate method of doing this is by directly measuring DNA synthesis. 5-Bromo-2'-deoxyuridine (BrdU) can be incorporated into the newly synthesized DNA of proliferating cells (during the S phase of the cell cycle) in place of thymidine. Our *In Situ* BrdU Cell Proliferation Assay Kit detects incorporated BrdU using an anti-BrdU antibody. This kit is designed for immunohistochemical staining of BrdU in formalin-fixed paraffin-embedded (FFPE) sections, frozen sections, and cultured or isolated cells on slides. Our anti-BrdU antibody works in all species tested (human, mouse, and rat) with strong specific staining and minimal background.

Materials required but not provided

- Xylene and ethanol
- Distilled or deionized water
- PBS, pH 7.4
- Fixative (3.7% formaldehyde in PBS)
- Permeabilization buffer (0.1% Triton® X-100 in PBS)
- 2 N HCl
- Phosphate/citric acid buffer, pH 7.4 (182 mL of 0.2 M Na_2HPO_4 + 18 mL 0.1 M citric acid)
- Mini PAP Pen
- Mounting Media

Experimental Protocols

BrdU Labeling

Prior to BrdU immunostaining for detection of proliferating cells, BrdU need to be incorporated into the living cells or tissues. Labeling can be done either *in vitro* or *in vivo* depending on the purpose of scientific research. The labeling concentration of BrdU varies depending on cells or tissue samples. A recommended starting concentration of BrdU for cell labeling is 10 µM.

1) *In vitro* BrdU Labeling

   To label cultured tissues or cells, BrdU is first prepared as 10 mM stock solution in DMSO, then dilute this stock solution in culture medium at a final concentration of 10 µM. For this step, do not disturb the cells in any way that may disrupt their normal cell cycling patterns. Incubate culture cells/tissues at 37 ºC for 1~4 hrs. The incubation time with BrdU is dependent on cells or tissue samples. Remove the culture medium and wash two times with PBS.

2) *In vivo* BrdU Labeling

Make a working solution of BrdU in PBS at 10 mg/ml. One to four hours before scarifying, mice are given intraperitoneally 70 mg BrdU/kg of body weight. Incorporation of BrdU can be detected in thymus and bone marrow in as little as 1 hr post injection. At 24 hr post injection BrdU can be detected in most tissues.

Fix, Permeabilize, and Denature

A. Cell Lines

1) Remove PBS and add 1 mL of fixative (3.7% formaldehyde in PBS).
2) Incubate for 15 minutes at room temperature.
3) Wash with PBS (3 times, 2 minutes each).
4) Remove PBS and add 1 mL of permeabilization buffer (0.1% Triton® X-100 in PBS).
5) Incubate for 20 minutes at room temperature.
6) Remove permeabilization buffer and add 1 mL of 2N HCl.
7) Incubate 10 minutes on ice, then 10 minutes at room temperature.
8) Remove acid solution and add 1 mL of phosphate/citric acid buffer, pH 7.4.
9) Incubate for 10 minutes at room temperature.
10) Wash with permeabilization buffer (3 times, 2 minutes each).

B. Frozen Sections

1) Snap frozen fresh tissues in liquid nitrogen or isopentane pre-cooled in liquid nitrogen, embedded in OCT compound in cryomolds. Store the frozen tissue block at -80°C until ready for sectioning.
2) Transfer the frozen tissue block to a cryotome cryostat (e.g. -20°C) prior to sectioning and allow the temperature of the frozen tissue block to equilibrate to the temperature of the cryotome cryostat.
3) Section the frozen tissue block into a desired thickness (typically 5-10 µm) using the cryotome.
4) Place the tissue sections onto glass slides suitable for immunohistochemistry.
5) Fix the tissue sections with ice cold acetone or ice cold methanol for 10 mintues. Air dry for 30 minutes.
6) Before staining, warm slides at room temperature for 30 minutes and fix in ice cold acetone or ice cold methanol for 10 minutes. Air dry for 30 minutes.
7) Add 1 mL of 2N HCl, and incubate 10 minutes on ice, then 10 minutes at room temperature.
8) Remove acid solution and add 1 mL of phosphate/citric acid buffer, pH 7.4.
9) Incubate for 10 minutes at room temperature.
10) Wash with permeabilization buffer (3 times, 2 minutes each).

C. Paraffin Sections

1) Deparaffinize sections in xylene (3 times, 5 minutes each).
2) Hydrate with 100% ethanol (2 times, 2 minutes each).
3) Hydrate with 95% ethanol (2 times, 2 minutes each).
4) Rinse with distilled water.
5) Add 1 mL of 2N HCl, and incubate 10 minutes on ice, then 10 minutes at room temperature.
6) Remove acid solution and add 1 mL of phosphate/citric acid buffer, pH 7.4.
7) Incubate for 10 minutes at room temperature.
8) Wash with permeabilization buffer (3 times, 2 minutes each).

Detecting Incorporated BrdU

1) Prepare 1X detection antibody solution by diluting BrdU Detection Antibody 1:100 with Antibody Diluent.
2) Prepare 1X Andy Fluor 488-conjugated secondary antibody solution by diluting Anti-mouse Andy Fluor 488-labeled Antibody 1:100 with Antibody Diluent.
3) Remove permeabilization buffer, and add 100 μL of 1X detection antibody solution.
4) Incubate at 4 °C for overnight or at RT for 1 hour in humidified chamber.
5) Wash with permeabilization buffer (3 times, 2 minutes each).
6) Remove permeabilization buffer, and add 100 μL of 1X Andy Fluor 488-conjugated secondary antibody solution.
7) Incubate at RT for 1 hour in humidified chamber.
8) Wash with PBS (3 times, 2 minutes each)..
9) Mount coverslip onto glass slide with anti-fade medium.
10) Image cells with appropriate filters.

References

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