

eBioscience™ BrdU Kit for IHC/ICC Colorimetric

Catalog Number: 8800-6599

RUO: For Research Use Only. Not for use in diagnostic procedures.

Product Information

Contents: eBioscience™ BrdU Kit for IHC/ICC Colorimetric
Catalog Number: 8800-6599
Handling Conditions: Use within 6 months of opening or by date indicated on the bottle

REF



Temperature Limitation: Refer to individual components

LOT

Batch Code: Refer to vial



Use By: Refer to vial



Contains sodium azide, formaldehyde, and hydrogen peroxide

Description

This BrdU Kit for immunohistochemistry (IHC)/immunocytochemistry (ICC) with colorimetric detection contains the necessary reagents and buffers for identifying and examining proliferating cells by immunohistochemical or immunocytochemical analysis. Cells are labeled in vitro or in vivo with 5-bromo-2'-deoxyuridine (BrdU), a synthetic analog of thymidine, which is incorporated into DNA in place of thymidine during the S-phase of the cell cycle. Following fixation and antigen retrieval steps, cells or tissue sections are stained for BrdU incorporation and visualized using an Avidin-HRP (horseradish peroxidase) and DAB (3, 3'-Diaminobenzidine) substrate reaction. This kit has been optimized for IHC with both frozen and paraffin embedded BrdU-labeled mouse intestine and ICC of BrdU-pulsed HeLa cells grown on culture slides.

Components

BrdU Fixation Buffer (cat. 00-8222-24): 15 mL; store at 2-8°C.

BrdU Antigen Retrieval Solution (10X) (cat. 00-4955-56): 100 mL; store at room temperature.

BrdU Blocking Solution (cat. 00-4952-52): 20 mL; store at 2-8°C.

Anti-BrdU Biotin (clone BU20A) (cat. 13-5071-63): 10 µg; store at 2-8°C.

Avidin-HRP(cat. IH18-4200-102): 60 µL; store at 2-8°C.

3, 3'-Diaminobenzidine (DAB) Substrate Solution: Reagent A (DAB Chromogen; 250 µL), Reagent B (DAB Diluent; 6 mL), and Dropper Bottle (for storage and application of working DAB solution) (cat. 8801-4965-72): store at 2-8°C.

Applications Reported

This BrdU Kit for IHC/ICC Colorimetric has been reported for use in immunohistochemical staining of frozen tissue sections, immunohistochemical staining of formalin-fixed paraffin embedded tissue sections, microscopy, and immunocytochemistry.

Applications Tested

This BrdU Kit for IHC/ICC Colorimetric has been tested by immunohistochemistry of formalin-fixed paraffin embedded and frozen BrdU-treated mouse intestine, and by immunocytochemistry of BrdU-pulsed HeLa cells.

Not for further distribution without written consent.

Copyright © 2016 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • thermofisher.com/ebioscience • info@ebioscience.com

BrdU Kit for IHC/ICC Colorimetric

Introduction

This BrdU Kit for immunohistochemistry (IHC)/immunocytochemistry (ICC) with colorimetric detection contains the necessary reagents and buffers for identifying and examining proliferating cells by immunohistochemical or immunocytochemical analysis. Cells are labeled *in vitro* or *in vivo* with 5-bromo-2'-deoxyuridine (BrdU), a synthetic analog of thymidine, which is incorporated into DNA in place of thymidine during the S-phase of the cell cycle. Following fixation and antigen retrieval steps, cells or tissue sections are stained for BrdU incorporation and visualized using an Avidin-HRP (horseradish peroxidase) and DAB (3, 3'-Diaminobenzidine) substrate reaction. This kit has been optimized for IHC with both frozen and paraffin embedded BrdU-labeled mouse intestine and ICC of BrdU-pulsed HeLa cells grown on culture slides.

Protocol

Materials Provided

- BrdU Fixation Buffer: Store at 2-8°C.
Note: This buffer contains formaldehyde. Avoid agitation. Avoid contact with skin, eyes, and mucous membranes.
- BrdU Antigen Retrieval Solution (10X): Store at room temperature.
- BrdU Blocking Solution: Store at 2-8°C.
- Anti-BrdU Biotin (clone BU20A): Store at 2-8°C.
- Avidin-HRP: Store at 2-8°C.
- DAB Substrate Solution: Reagent A (DAB Chromogen), Reagent B (DAB Diluent), and Dropper Bottle (for storage and application of working DAB solution): Store at 2-8°C.
Note: Reagent A is a suspected carcinogen. Reagent B contains hydrogen peroxide. For both Reagent A and B, avoid contact with skin and eyes and dispose in accordance with local regulations.

Additional Reagents Required

- BrdU
- Phosphate Buffer Saline (PBS) without sodium azide (137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.76 mM KH₂PO₄, pH 7.4)
- Humidified Chamber
- HistoClear II or equivalent
- Ethanol (100%, 90%, and 70%)
- 30% Hydrogen Peroxide (H₂O₂) Stock Solution
- Hematoxylin
- Permount
- Parafilm

Experimental Procedure

Step I: BrdU Labeling

In vitro labeling of cells with BrdU

1. Under sterile conditions, thaw BrdU on ice and dilute to a working concentration of 1 mM with sterile 1X PBS. Add 10 μM BrdU to each sample. (For example, add 10 μL of 1 mM BrdU directly to every milliliter of tissue culture medium.)
2. Incubate cells long enough to allow incorporation of BrdU. Incubation time will be dependent on specific culture conditions and the proliferation kinetics of the cell type, to be determined empirically. After incubation, proceed with immunostaining protocol for cultured cells or cytopins.

In vivo labeling of mouse tissues with BrdU

- **Intraperitoneal Injection:** Dilute BrdU to a working concentration of 1 mg in 200 μL of sterile PBS (5 mg/mL). Inject mice intraperitoneally with 200 μL (1 mg) of BrdU solution. Incorporation of BrdU in the small intestine can be detected within 1 hour of injection. BrdU incorporation can be detected in most tissues within 24 hours of injection. Optimal incubation times should be determined for the tissues of interest.
- **Drinking Water:** Dilute BrdU to 0.8 mg/mL in drinking water. This solution must be prepared fresh and changed daily. Optimal incorporation times should be determined for the tissues of interest. Prolonged feeding of BrdU can have toxic and even lethal effects.

Step II: Tissue Processing

Formalin-fixed paraffin embedded tissues

1. Harvest the tissue and rinse in PBS. Trim and cut tissue to the appropriate size (generally less than 1 mm thickness). Some tissues may require additional preparation before fixation (such as flushing of the intestine).
2. Fix tissue in 10% neutral buffered formalin for ≥ 48 hours at room temperature for 1 mm thick tissues.
3. Wash 2x15 minutes in deionized water.
4. Dehydrate tissues using a tissue processor or manually using the following sequence:
 - a. 70% Ethanol 1x20 minutes
 - b. 90% Ethanol 2x20 minutes
 - c. 100% Ethanol 2x20 minutes
 - d. HistoClear II 2x20 minutes
 - e. Paraffin at 65°C for 2 hours
5. Embed tissues in paraffin blocks. Tissue blocks can be stored at room temperature.
6. Tissues can be sectioned at 3-10 μm . Store slides at room temperature. For best results slides should be stained promptly.
7. Proceed with immunostaining protocol for formalin-fixed paraffin embedded sections.

Frozen tissues

1. Harvest the tissue and rinse in PBS. Trim and cut tissue to the appropriate size (less than 1 mm thickness). Some tissues may require additional preparation before flash freezing (such as flushing of the intestine).
2. Flash freeze tissue using cold 2-methylbutane.
3. Mount tissue in OCT embedding compound in a cryomold.
4. Freeze on dry ice. Store tissue blocks at -20 to -80°C.
5. Tissues can be sectioned at up to 10 μm using a cryostat. Store slides at -20 to -80°C. For best results slides should be stained promptly.
6. Proceed with immunostaining protocol for frozen sections.

Step III: Immunostaining for BrdU

Formalin-fixed paraffin embedded sections

1. Remove paraffin and rehydrate tissues using the following slide wash/incubation sequence:
 - a. HistoClear II 3x5 minutes
 - b. 100% Ethanol 2x5 minutes
 - c. 90% Ethanol 1x5 minutes
 - d. 70% Ethanol 1x5 minutes
 - e. PBS 2x5 minutes

NOTE: it is critical from this point on that the tissue does not dry out as this will result in high levels of background staining and difficulty in interpretation of staining results.

2. Block endogenous peroxidase with 0.3% hydrogen peroxide in PBS for 10 minutes.
3. Wash slides in PBS 3x5 minutes.
4. Continue with antigen retrieval and staining for BrdU as described below.

Frozen sections

1. Allow slides to come to room temperature.
2. Fix frozen sections with BrdU fixation buffer for 15 minutes at room temperature.

NOTE: it is critical from this point on that the tissue does not dry out as this will result in high levels of background staining and difficulty in interpretation of staining results.
3. Wash slides in PBS 2x5 minutes.
4. Block endogenous peroxidase with 0.3% hydrogen peroxide in PBS for 10 minutes.
5. Wash slides in PBS 3x5 minutes.
6. Continue with antigen retrieval and staining for BrdU as described below.

Cultured cells or cytopins

1. Fix cells cultured on culture slides or on cytopins with BrdU fixation buffer for 15 minutes.

NOTE: it is critical from this point on that the tissue does not dry out as this will result in high levels of background staining and difficulty in interpretation of staining results.
2. Wash slides in PBS 2x5 minutes.
3. Block endogenous peroxidase with 0.3% hydrogen peroxide in PBS for 10 minutes at room temperature.
4. Wash slides in PBS 3x5 minutes.
5. If using culture chamber slides, remove plastic chamber at this time.
6. Continue with antigen retrieval and staining for BrdU as described below.

Antigen retrieval and staining for BrdU

1. Prepare 1X antigen retrieval solution by diluting 10X BrdU Antigen Retrieval Solution with deionized water.
2. Place slides in a heat safe container (such as a plastic coplin jar or glass beaker) in 1X antigen retrieval solution in water bath at 97°C for 15 minutes. Remove container from water bath and allow slides to cool in the solution to room temperature for a minimum of 20 minutes and up to 1 hour.
3. Wash slides in 1X PBS 3x5 minutes.
4. Block slides with approximately 100 µL 1X BrdU Blocking Solution for 10 minutes at room temperature. Volume may vary depending on size of the tissue. To minimize buffer needed, limit evaporation of blocking reagent and to help evenly spread the blocking solution, gently overlay the tissue section/chamber slide with a piece of Parafilm cut to the dimension of the tissue. The Parafilm is not stretched and should only contact the blocking solution and not touch the tissue directly. Ensure that there are no air bubbles trapped underneath the Parafilm layer. Incubate slides in a humidified container to further reduce the amount of evaporation.
5. Dilute the biotinylated Anti-BrdU antibody to 1 µg/mL in 1X BrdU Blocking Solution (for one slide, dilute 0.2 µL of antibody in 100 µL 1X BrdU Blocking Solution). Calculate amount of antibody required and dilute fresh for each experiment. Apply the antibody to the sections on the slide (100 µL/slide) and cover with parafilm. Incubate for a minimum of 2 hours at room temperature or overnight at 4°C in a humidified container. Note: maximum intensity of staining is obtained with overnight incubation.
6. Wash slides in 1X PBS 3x2 minutes.
7. Dilute the Avidin-HRP 1:100 in 1X BrdU Blocking Solution. Calculate amount of Avidin-HRP required and dilute fresh for each experiment. Apply the Avidin-HRP (typically 100 µL) to the sections/chamber on the slide and cover with parafilm. Incubate for 1 hour at room temperature in a humidified container.
8. Wash slides in 1X PBS 3x2 minutes.
9. Prepare DAB substrate solution by adding 20 µL Reagent A (DAB chromogen) to 500 µL Reagent B (DAB Diluent). Apply DAB substrate solution to cover the tissue section and incubate until the desired color intensity develops (up to 20 minutes).
10. Rinse slides in deionized water 3x2 minutes.
11. Counterstain slides in hematoxylin (optional).
12. Rinse thoroughly in deionized water. Dehydrate slides in reverse order of hydration:
 - a. 70% Ethanol 1x5 minutes
 - b. 90% Ethanol 1x5 minutes
 - c. 100% Ethanol 2x5 minutes
 - d. Histoclear II 3x5 minutes
13. Mount and coverslip using 3 drops of Permount. Allow slides to dry for 1-2 hours before visualizing using a brightfield microscope.

Documentation and support

Customer and technical support

Visit thermofisher.com/support for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
 - Product FAQs
 - Software, patches, and updates
- Order and web support
- Product documentation, including:
 - User guides, manuals, and protocols
 - Certificates of Analysis
 - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at thermofisher.com/support.

The information in this guide is subject to change without notice.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Corporate entity: Life Technologies | Carlsbad, CA 92008 USA | Toll Free in USA 1.800.955.6288

©2017 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. All other trademarks are properties of their respective owners.

For support visit thermofisher.com/support or email techsupport@lifetech.com

thermofisher.com

23 January 2017

ThermoFisher
SCIENTIFIC