eBioscience™ BrdU Kit for IHC/ICC Immunofluorescence eFluor™ 570

Catalog Number: 8841-6599
RUO: For Research Use Only. Not for use in diagnostic procedures.

Product Information

Contents: eBioscience™ BrdU Kit for IHC/ICC Immunofluorescence eFluor™ 570
REF **Catalog Number:** 8841-6599
Handling Conditions: Use within 6 months of opening or by date indicated on the bottle

Temperature Limitation: Refer to individual components
LOT **Batch Code:** Refer to vial
Use By: Refer to vial
Contains sodium azide and formaldehyde

Description

This BrdU Kit for immunohistochemistry (IHC)/immunocytochemistry (ICC) with immunofluorescence 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 a streptavidin-conjugated fluorophore. This kit has been optimized for IHC with both frozen and formalin-fixed 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.
Streptavidin for BrdU IHC/ICC eFluor® 570 (cat. 41-4318-61): 2 µg; store at 2-8°C.

Applications Reported

This BrdU Kit for IHC/ICC Immunofluorescence eFluor® 570 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 Immunofluorescence eFluor® 570 has been tested by immunohistochemistry of formalin-fixed paraffin embedded and frozen BrdU-treated mouse intestine, and by immunocytochemistry of BrdU-pulsed HeLa cells.

Related Products

00-4959 Fluoromount-G™, with DAPI
44-0404 eBioscience™ StainTray™

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invitrogen

BrdU Kit for IHC/ICC Immunofluorescence

Introduction

This BrdU Kit for immunohistochemistry (IHC)/immunocytochemistry (ICC) with immunofluorescence 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 a streptavidin-conjugated fluorophore. This kit has been optimized for IHC with both frozen and formalin-fixed paraffin embedded BrdU-labeled mouse intestine and ICC of BrdU-pulsed HeLa cells grown on culture slides.

Protocol: BrdU Kit for IHC/ICC Immunofluorescence

Materials Provided

- BrdU Fixation Buffer: Store at 2-8°C.
  Note: This buffer contains formaldehyde. Avoid contact with skin, eyes, and mucous membranes. Avoid agitation.
- 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.
- Streptavidin conjugated to eFluor™ 570, eFluor™ 615, or eFluor™ 660: Store at 2-8°C.

Additional Reagents Required

- BrdU
- Phosphate Buffer Saline (PBS) (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.76 mM KH_2PO_4, pH 7.4)
- Humidified Chamber
- Histoclear II, or equivalent
- Ethanol (100%, 90%, and 70%)
- Fluoromount-G with or without DAPI (Cat. No. 00-4959 or 00-4958)
- Parafilm
- OCT embedding compound
- 2-methlybutane
- Triton X-100

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 cytospins.

In vivo labeling of mouse tissues with BrdU by intraperitoneal injection

1. Dilute BrdU to a working concentration of 1 mg in 200 µL of sterile PBS (5 mg/mL).
2. 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.

In vivo labeling of mouse tissues with BrdU through drinking water

1. 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 um 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 3x5 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. 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 (approximately 100 μL/slide).

   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 3x5 minutes.
4. Continue with antigen retrieval and staining for BrdU as described below.

Cultured cells or cytospins

1. Fix cells cultured on culture slides or on cytospins with BrdU fixation buffer for 15 minutes (approximately 100 µL/slide).
   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. Permeabilize with 1% Triton X in PBS for 30 minutes at 37°C.
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 (approximately 50 mL of 1X antigen retrieval solution required per plastic coplin jar).
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/cells on the slide (approximately 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 streptavidin conjugate in 1X BrdU Blocking Solution. Calculate amount of streptavidin conjugate required (approximately 100 µL/slide) and dilute fresh for each experiment (for one slide, dilute 2.5 µL of antibody in 97.5 µL 1X BrdU Blocking Solution). Apply the streptavidin conjugate to the sections/cells on the slide and cover with parafilm. Incubate for 30 minutes at room temperature in a humidified container.
8. Wash slides in 1X PBS 3x2 minutes.
9. Mount and coverslip using 2-3 drops Fluoromount-G mounting medium with or without DAPI. Seal coverslip with clear nail polish and allow to dry for 20 minutes in the dark.
10. For best results, image slides immediately. Slides can be stored at 4°C protected from light.

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