For life science research only.
Not for use in diagnostic procedures.

5-Bromo-2'-deoxy-uridine Labeling and Detection Kit II

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Immunohistocytochemical assay for the detection of 5-bromo-2'-deoxy-uridine (BrdU) incorporated into cellular DNA

Cat. No. 11 299 964 001 1 kit
100 tests

Store the kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	red	5-Bromo-2′-deoxy-uridine Labeling and Detection Kit II, BrdU Labeling reagent, 1,000x conc.	▪ 10 mM 5-bromo-2′-deoxy-uridine in PBS, pH 7.4. ▪ Filtered through 0.2 µm pore-size membrane. ▪ For labeling of DNA.	1 bottle, 10 ml
2	colorless	5-Bromo-2′-deoxy-uridine Labeling and Detection Kit II, Washing buffer, 10x conc.	▪ Phosphate-buffered saline (PBS) ▪ For wash steps.	1 bottle, 100 ml
3	green	5-Bromo-2′-deoxy-uridine Labeling and Detection Kit II, Incubation buffer	▪ 66 mM Tris buffer, 0.66 mM $MgCl_2$, 1 mM 2-mercaptoethanol. ▪ For preparation of the BrdU Working solution.	1 bottle, 100 ml
4	yellow	5-Bromo-2′-deoxy-uridine Labeling and Detection Kit II, Anti-BrdU	▪ Monoclonal antibody from mouse (clone BMG 6H8 IgG_1) containing nucleases for DNA denaturation, in PBS/glycerin. ▪ For the binding of the BrdU incorporated into the DNA.	1 bottle, 1 ml
5	blue	5-Bromo-2′-deoxy-uridine Labeling and Detection Kit II, Anti-mouse-Ig-AP	▪ From sheep. ▪ Immunosorptively purified in triethanolamine buffer. ▪ For the binding of the BrdU antibody.	1 bottle, 1 ml
6	white	5-Bromo-2′-deoxy-uridine Labeling and Detection Kit II, NBT	▪ Nitroblue tetrazolium (NBT) salt in 70% dimethylformamide (v/v). ▪ 75 mg/ml ▪ For the detection of alkaline phosphatase.	1 bottle, 1.5 ml
7	violet	5-Bromo-2′-deoxy-uridine Labeling and Detection Kit II, X-phosphate	▪ 5-bromo-4-chloro-3-indolyl phosphate, toluidinium in dimethylformamide (BCIP) ▪ 50 mg/ml ▪ For the detection of alkaline phosphatase.	1 bottle, 1.2 ml

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the kit is stable through the expiration date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	red	BrdU Labeling reagent, 1,000x conc.	Store at −15 to −25°C.
2	colorless	Washing buffer, 10x conc.	
3	green	Incubation buffer	
4	yellow	Anti-BrdU	
5	blue	Anti-mouse-Ig-AP	Store at −15 to −25°C. ⚠ ***Once opened, store at +2 to +8°C.***
6	white	NBT	Store at −15 to −25°C.
7	violet	X-phosphate	

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Coverslips or chamber slides
- +37°C, 5% CO_2 incubator
- Light microscope
- Humidified chamber
- Sharp blade
- Cryostat
- Ultramicrotome

For immunocytochemistry of adherent cells

- Ethanol fixative
  See section, **Working Solution** *for preparation of fixative.*
- Mounting medium, such as Kaiser's Glycerol Gelatin

For immunocytochemistry of suspension cells or cell smear preparations

- Cytocentrifuge for cytospin preparations
- Poly-L-lysine-coated glass slides, fat-free
- PBS*
- 5% albumin
- Ethanol fixative
  See section, **Working Solution** *for preparation of fixative.*
- Cellulose cloth
- Mounting medium, such as Kaiser's Glycerol Gelatin

For immunohistochemistry of tissue sections

- Poly-L-lysine-coated glass slides, fat-free
- Cellulose cloth
- Mounting medium, such as Kaiser's Glycerol Gelatin

Additional reagents

- Sterile cell culture medium
- Double-distilled water
- Tris-HCl*

1.4. Application

The kit can be used for the detection of BrdU incorporated into cellular DNA by immunohistocytochemistry.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

The Labeling and Detection Kit II can be used with a variety of samples:
- Adherent and suspension cells.
- Organ or explant tissues.
- Frozen or paraffin-embedded tissue sections after *in vivo* labeling.

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

Solution	Preparation	Storage and Stability	For use in...
BrdU labeling solution	▪ Dilute BrdU Labeling reagent (Bottle 1) 1:1,000 with sterile cell culture medium (final concentration: 10 µM BrdU). *For in vivo labeling, use undiluted BrdU Labeling reagent (1 to 2 ml/100 g body weight).*	⚠ ***Prepare shortly before use.*** Store the undiluted BrdU Labeling reagent, 1,000x conc. (Bottle 1) in aliquots at −15 to −25°C.	Cell labeling.
Anti-BrdU working solution	▪ Dilute Anti-BrdU stock solution (Bottle 4) 1:10 with Incubation buffer (Bottle 3).	⚠ ***Prepare shortly before use.*** Store undiluted antibody at −15 to −25°C.	Binding to incorporated BrdU.
Anti-mouse-Ig-AP working solution	▪ Dilute Anti-mouse-Ig-AP solution (Bottle 5) 1:10 with PBS.	⚠ ***Prepare shortly before use.***	Binding to Anti-BrdU antibody.
Washing buffer	▪ Dilute Washing Buffer, 10x conc. (Bottle 2) 1:10 with double-distilled water.	Store at +2 to +8°C.	Removal of unbound antibodies.
Color substrate solution	▪ 13 µl NBT solution (Bottle 6) and 10 µl X-phosphate solution (Bottle 7) are added to 3 ml Substrate buffer.	⚠ ***Prepare shortly before use.***	Visualization of antibody binding.
Ethanol fixative	▪ Add 50 mM glycine solution to 70 ml absolute ethanol to obtain 100 ml fixative, pH 2.0.	Store at +2 to +8°C.	Fixation of cells.
Substrate buffer	▪ Prepare 100 mM Tris-HCl buffer, 100 mM NaCl, 50 mM $MgCl_2$, pH 9.5 (+20°C).	Store at +2 to +8°C.	Preparation of color substrate solution.

2.2. Protocols

Immunocytochemistry using adherent cells

See section, **Working Solution** *for additional information on preparing solutions.*

1. Grow cells on coverslips or chamber slides until they have reached approximately 50% confluency.

2. Aspirate cell culture medium and add BrdU labeling solution.

3. Incubate the cells at +37°C and 5% CO_2 for approximately 15 to 60 minutes.
   The incubation time depends on the cells used and the individual requirements.

4. Aspirate the BrdU labeling solution.

5. Wash the coverslips three times in Washing buffer.

6. Fix the cells with the Ethanol fixative for at least 20 minutes at −15 to −25°C.

7. Wash the coverslips three times in Washing buffer.

8. Cover the cells with Anti-BrdU working solution.
   – Incubate for 30 minutes at +37°C.

9. Wash the coverslips three times in Washing buffer.

10. Cover the cells with Anti-mouse-Ig-AP solution.
    – Incubate for 30 minutes at +37°C.

11. Wash the coverslips three times in Washing buffer.

12. Cover the cells with a sufficient amount of freshly prepared color Substrate solution.
    – Incubate for 15 to 30 minutes at +15 to +25°C.

13. For immediate analysis, place a coverslip onto cell-carrying coverslip or chamber slide, and evaluate using a light microscope.
    – Alternatively, cover the preparations with an appropriate mounting medium, such as Kaiser's glycerol gelatin.

14. Examine using a light microscope.

Immunocytochemistry using suspension cells by cytocentrifugation or cell smear preparation

See section, **Working Solution** *for additional information on preparing solutions.*

1. Centrifuge the cell suspension at 300 × *g* for 5 to 10 minutes and aspirate the supernatant (cell culture medium).

2. Add BrdU labeling solution (0.5 ml/10^6 cells) and resuspend the cells.

3. Incubate the cell suspension for 15 to 60 minutes at +37°C and 5% CO_2.

   The incubation period depends on the cell type and the individual requirements.

4. Add Washing buffer to the cells.
   – Centrifuge the cell suspension at 300 × *g* for approximately 5 minutes.
   – Carefully remove supernatant.

5. Repeat Step 4 two additional times.

6. Prepare cytospins and cell smears as shown in the following table:

Cytospin	Cell Smear
Centrifuge 100 µl of the labeled cell suspension (3 × 10^5 cells/ml, resuspended in PBS/5% albumin) onto a clean, fat-free, poly-L-lysine-coated glass slide using a cytocentrifuge.	Place 1 drop, approximately 5 to 10 µl of the labeled cell suspension (5 × 10^7 cells/ml, resuspended in PBS/5% albumin) on one end of a clean, fat-free, poly-L-lysine-coated glass slide. – Smoothly and evenly, push a second glass slide across the length of the first slide, drawing the liquid in a film over the slide. – Allow samples to air dry at +15 to +25°C.

7. Fix the cells with the Ethanol fixative for at least 20 minutes at −15 to −25°C.

8. Wash glass slides with cells 3 times with Washing buffer.
   – Carefully dry the peripheral zone of the area to be stained, for example, with a cellulose cloth.

9. Cover the cells with a sufficient amount of Anti-BrdU working solution.
   – Incubate glass slides for 30 minutes at +37°C in a humidified atmosphere.

10. Wash glass slides with cells 3 times with Washing buffer.
    – Carefully dry the peripheral zone of the area to be stained, for example, with a cellulose cloth.

11. Cover the cells with a sufficient amount of Anti-mouse-Ig-AP working solution.
    – Incubate the glass slide for 30 minutes at +37°C in a humidified atmosphere.

12. Wash glass slides with cells 3 times with Washing buffer.
    – Carefully dry the peripheral zone of the area to be stained, for example, with a cellulose cloth.

13. Cover the cells with a sufficient amount of freshly prepared color Substrate solution.
    – Incubate at +15 to +25°C for 15 to 30 minutes.

14. Carefully remove color Substrate solution by washing with a sufficient amount of Washing buffer; carefully dry the peripheral zone of the area to be stained.

15. Cover the preparations with an appropriate mounting medium, such as Kaiser's glycerol gelatin.

16. Examine using a light microscope.

Immunohistochemistry using frozen or paraffin-embedded tissue sections

Labeling with BrdU *in vivo*

1. Inject animal intravenously with undiluted BrdU Labeling reagent (Bottle 1), 1 to 2 ml/100 g body weight.

2. Sacrifice animal 1 hour after injection and remove selected organs.

3. Process tissue for frozen sectioning or paraffin-embedding.

Labeling of tissue slices

See section, **Working Solution** *for additional information on preparing solutions.*

1. Place tissue sample in +37°C pre-warmed cell culture medium.

2. Cut tissue sample with a sharp blade to obtain thin slices, approximately 1 mm thick and 2 mm^2 in area.

3. Aspirate cell culture medium and add a sufficient amount of BrdU labeling solution.
   – Incubate for 30 to 60 minutes at +37°C and 5% CO_2.

   The incubation period depends on the tissue type used and the individual requirements.

4. Remove labeling medium and add Washing buffer to the tissue slices.
   – Incubate for 25 minutes at +37°C and 5% CO_2.

5. Process tissue slices for frozen sectioning or paraffin-embedding.

Preparation of frozen sections

1. Prepare 3 to 5 µm thick frozen tissue sections in a cryostat.

2. Apply sections directly onto clean, fat-free, poly-L-lysine-coated glass slides.
   – Air dry most tissues at +15 to +25°C prior to further use.

3. Fix sections with the Ethanol fixative for at least 20 minutes at −15 to −25°C.

Preparation of paraffin-embedded sections

1. Prepare 3 to 5 µm thick paraffin-embedded sections in a ultramicrotome.

2. Thoroughly dewax sections prior to further use.

Immunohistochemical procedure

See section, **Working Solution** *for additional information on preparing solutions.*

1. Rehydrate frozen or paraffin-embedded tissue sections by washing 3 times with Washing buffer.
   – Carefully dry the peripheral zone of the area to be stained, for example, with a cellulose cloth.

2. Cover the section with a sufficient amount of Anti-BrdU working solution.
   – Incubate glass slides for 30 minutes at +37°C in a humidified atmosphere.

3. Wash glass slides 3 times with Washing buffer.
   – Carefully dry the peripheral zone of the area to be stained, for example, with a cellulose cloth.

4. Cover the sections with a sufficient amount of Anti-mouse-Ig-AP working solution.
   – Incubate the glass slides for 30 minutes at +37°C in a humidified atmosphere.

5. Wash glass slides 3 times with Washing buffer.
   – Carefully dry the peripheral zone of the area to be stained, for example, with a cellulose cloth.

6. Cover the the cells with a sufficient amount of freshly prepared color Substrate solution.
   – Incubate at +15 to +25°C for 15 to 30 minutes.

7. Remove color Substrate solution carefully by washing with a sufficient amount of Washing buffer.
   – Carefully dry peripheral zone of the area to be stained.

8. Cover the preparations with an appropriate mounting medium, such as Kaiser's glycerol gelatin.

9. Examine using a light microscope.

2.3. Parameters

Specificity

Anti-BrdU monoclonal antibody specifically binds to 5-bromo-2′-deoxy-uridine, and shows cross-reactivity with 5-iodo-2′-deoxy-uridine (10%). Anti-BrdU shows no cross-reactivity with 5-fluoro-2′-deoxy-uridine or any endogenous cellular component, such as thymidine or uridine.

3. Additional Information on this Product

3.1. Test Principle

Assay overview

1. Cells, tissue explants, or organ cultures are incubated with 10 µmol BrdU for a short period of time, approximately 30 minutes.
   – The addition of 5′-fluoro-2′-deoxy-uridine (FdU), described to enhance BrdU incorporation, has no advantage within short incubation periods and BrdU concentrations of 10 µM.

2. Fixation of samples with ethanol.

3. Incubation with anti-BrdU monoclonal antibody.
   – The monoclonal antibody binds to BrdU incorporated into cellular DNA.

4. Incubation with anti-mouse-Ig-alkaline phosphatase.

5. Color reaction of NBT/BCIP with the alkaline phosphatase.

6. Bound anti-BrdU monoclonal antibody is visualized by light microscopy.

Measurement of DNA synthesis

The ability to measure DNA synthesis or cell proliferation is important in cell biology research. The measurement of cell proliferation or DNA synthesis by determining the incorporation of [^{3}H] thymidine into cellular DNA has become a widely used assay. [^{3}H]-thymidine incorporation into DNA is detected by autoradiography. Because this assay is labor intensive and uses expensive and potentially hazardous materials, alternative assays have been developed. 5-bromo-2′-deoxy-uridine (BrdU) can be incorporated into DNA in place of thymidine, and monoclonal antibodies directed against BrdU have been developed. Cells which have incorporated BrdU into DNA can be quickly detected using a monoclonal antibody against BrdU and an enzyme- or fluorochrome-conjugated second antibody.

How this product works

Normally, binding of the antibody is only achieved by denaturation of the DNA. This is usually obtained by exposing the cells to acid, base, or heat. These procedures result in the destruction of cell integrity, including cell morphology, and surface and cytoplasmic markers. The BrdU Labeling and Detection Kit II avoids these problems. The antibody preparation contains specific nucleases which allows access to BrdU after fixation in acidic ethanol, allowing simultaneous detection of other markers (double staining).

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Editorial changes.
Change the lid color of bottle 5 of the kit from brown to blue.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Tris hydrochloride	500 g	10 812 846 001
Buffers in a Box, Premixed PBS Buffer, 10x	4 l	11 666 789 001

4.4. Trademarks

All product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products and select the corresponding product catalog.

4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed

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