

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

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

Cat. No. 11 299 964 001

Kit for 100 tests

Version September 2007

Store at -15 to -25°C

## 1. Preface

### 1.1 Kit contents

Vial	Label	Content including function
1 red cap	BrdU labeling reagent (1000× conc.)	• 1 × 10 ml BrdU stock solution (1,000 × conc.) in 10 mM phosphate buffered saline (PBS), sterile for the labeling of DNA
2 colorless cap	Washing buffer concentrate (10× conc.)	• 1 × 100 ml PBS (10 × conc.) • for wash steps
3 green cap	Incubation buffer	• 1 × 100 ml [66 mM Tris buffer, 0.66 mM MgCl <sub>2</sub> , 1 mM 2-mercaptoethanol] • for the preparation of the BrdU working solution
4 yellow cap	Anti-BrdU with nucleases	• 1 × 1 ml anti-BrdU, mouse monoclonal antibody (clone BMG 6H8) containing nucleases for DNA denaturation, in PBS/glycerin • binding of in the DNA incorporated BrdU
5 brown cap	Anti-mouse Ig-alkaline phosphatase	• 1 × 1 ml anti-mouse Ig-alkaline phosphatase (AP) from sheep, immunosorptively purified, in triethanolamine buffer • binding of the BrdU antibody
6 white cap	NBT	• 1 × 1.5 ml nitroblue tetrazolium (NBT) salt in 70% dimethylformamide (v/v) • [75 mg/ml] • for the detection of the alkaline phosphatase
7 violet cap	BCIP	• 1 × 1.2 ml 5-bromo-4-chloro-3-indolyl phosphate, toluidinium in dimethylformamide (BCIP)* • [50 mg/ml] • for the detection of the alkaline phosphatase

## 2. Introduction

### 2.1 Product overview

#### General

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 [<sup>3</sup>H]-thymidine into cellular DNA has become a widely used assay.

[<sup>3</sup>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. Monoclonal antibodies directed against BrdU have been developed (1-3).

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.

### Assay principle

Cells, tissue explants or organ cultures are incubated *in vitro* with BrdU, 10 μmol, for a short period of time (approx. 30 min). The addition of 5-fluoro-2'-deoxy-uridine (FdU), being described to enhance BrdU incorporation has no advantage within short incubation periods and BrdU concentrations of 10 μmol/l (4).

The samples are fixed with ethanol and incubated with anti-BrdU monoclonal antibody. The monoclonal antibody binds to BrdU incorporated into cellular DNA. 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 destruction of cell integrity including cell morphology and surface and cytoplasmatic 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 ethanol. Therefore also simultaneous detection of other markers (double staining) is possible. After incubation with anti-mouse-Ig-alkaline phosphatase followed by the substrate reaction, bound anti-BrdU monoclonal antibody is visualized by light microscopy.

Stage	Description
1	Incorporation of BrdU in DNA.
2	Binding of anti-BrdU antibody to the in DNA incorporated BrdU.
3	Binding of the AP conjugated anti-mouse-antibody to the anti-BrdU antibody.
4	Color reaction of NBT/BCIP with the alkaline phosphatase and detection by light microscopy.

### Application

The kit is used for the detection of BrdU incorporated into cellular DNA by immunocyto/histochemistry.

### Sample material

- Cell culture:
  - adherent cells
  - suspension cells
  - organ or explant tissues
- Frozen or paraffin-embedded tissue sections (after *in vivo* labeling)

### Number of tests

The kit is designed for 100 tests.

### Kit storage/stability

The unopened kit is stable at -15 to -25°C until the expiration date printed on the label.

**Note:** Once opened, the AP-conjugate (bottle 5) should be stored at +2 to +8°C.

### 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 either with 5'-fluoro-2'-deoxy-uridine, or with any endogenous cellular components, such as thymidine or uridine.

### 3. Procedures and required materials

#### 3.1 Before you begin

##### Additional reagents required

- Sterile cell culture medium
- Double dist. water
- PBS/5% albumin (needed for cytospin- and cell smear preparations)
- Mounting medium (e.g., Kaiser's glycerin-gelatin)

##### Additional equipment required

- Fat-free, poly-L-lysine coated glass slides (needed for cytospin- and cell smear preparations and for preparation of frozen sections)
- Cyto centrifuge (needed for cytospin preparations)

##### Additional working solutions required

The following additional solutions are required for the assays:

Solution	Composition/preparation	Storage and stability	Use
Ethanol fixative	Add 50 mM glycine solution to 70 ml abs. EtOH to get 100 ml fixative, pH 2.0.	Stable at +2 to +8°C	Fixation of cells
Substrate buffer	Prepare 100 mM Tris HCl-buffer, 100 mM NaCl, 50 mM MgCl <sub>2</sub> , pH 9.5 (20°C).	Stable at +2 to +8°C	Preparation of Color substrate solution

##### Preparation of kit working solutions

The following table shows the preparation of kit working solutions:

Solution	Composition/preparation	Storage and stability	Use
BrdU labeling medium	Dilute BrdU labeling reagent (bottle 1) 1:1000 with sterile cell culture medium (final concentration 10 µmol/l BrdU). <b>Note:</b> For <i>in vivo</i> labeling undiluted BrdU labeling reagent (1–2 ml/100 g body weight) is needed.	Prepare shortly before use.	<i>In vitro</i> labeling
Anti-BrdU working solution	Dilute anti-BrdU solution (bottle 4) 1:10 with Incubation buffer (bottle 3).	Prepare shortly before use.	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 the anti-BrdU antibody
Washing buffer	Dilute Washing buffer concentrate (10×) (bottle 2) 1:10 with double dist. water.	Stable at +2 to +8°C	Washing purposes
Color-substrate solution	13 µl NBT-solution (bottle 6) and 10 µl BCIP-solution (bottle 7) are added to 3 ml Substrate buffer.	Prepare shortly before use.	Visualization of antibody binding

### 3.2 Immunocytochemistry using adherent cells

#### Procedure

Please refer to the following table:

Step	Action
1	Grow cells on cover slips (or chamber slides) until they have reached about 50% confluency.
2	Aspirate cell culture medium and add BrdU labeling medium.
3	Incubate the cells at 37°C, 5% CO <sub>2</sub> for about 15–60 min. <b>Note:</b> The incubation time depends on the cells used and the individual requirements.
4	Aspirate the BrdU labeling medium.
5	Wash the cover slips three times in Washing buffer.
6	Fix the cells with the Ethanol fixative for at least 20 min at –15 to –25°C.
7	Wash the cover slips three times in Washing buffer.
8	<ul style="list-style-type: none"> <li>• Cover the cells with Anti-BrdU working solution.</li> <li>• Incubate for 30 min at 37°C.</li> </ul>
9	Wash the cover slips three times in Washing buffer.
10	<ul style="list-style-type: none"> <li>• Cover the cells with Anti-mouse-Ig-AP solution.</li> <li>• Incubate for 30 min at 37°C.</li> </ul>
11	Wash the cover slips three times in Washing buffer.
12	<ul style="list-style-type: none"> <li>• Cover the cells with a sufficient amount of freshly prepared Color-substrate solution.</li> <li>• Incubate for 15–30 min at +15 to +25°C.</li> </ul>
13	For immediate analysis put a coverslip onto cell-carrying coverslip (or chamber slide) and evaluate in a light microscope.
14	Alternatively cover the preparations with an appropriate mounting medium (e.g., Kaiser's glycerin-gelatin).
15	Examine in a light microscope.

### 3.3 Immunocytochemistry using suspension cells by cytospin- or cell smear preparation

#### Procedure

Please refer to the following table.

Step	Action
1	Centrifuge the cell suspension at $300 \times g$ for 5–10 min and aspirate the supernatant (cell culture medium).
2	Add BrdU labeling medium ( $0.5 \text{ ml}/10^6$ cells) and resuspend the cells.
3	Incubate the cell suspension for 15 – 60 min at $37^\circ\text{C}$ , $5\% \text{ CO}_2$ . <b>Note:</b> The incubation period depends on the cell type and the individual requirements.
4	<ul style="list-style-type: none"> <li>• Add Washing buffer to the cells.</li> <li>• Spin cell suspension down (5 min, <math>300 \times g</math>).</li> <li>• Remove supernatant carefully.</li> </ul>
5	Repeat washing 2x as described under 4.
6a	Preparing of cytospin-preparations: Centrifuge $100 \mu\text{l}$ of the labeled cell suspension ( $3 \times 10^9$ cells/ml, resuspended in PBS/5% albumin) onto a clean, fat-free, poly-L-lysine coated glass slide with a cytocentrifuge.
6b	Preparing of cell smears: <ul style="list-style-type: none"> <li>• Place 1 drop (approx. <math>5\text{--}10 \mu\text{l}</math>) of the labeled cell suspension (<math>5 \times 10^7</math> cells/ml, resuspended in PBS/5% albumin) on one end of a clean, fat-free, poly-L-lysine coated glass slide.</li> <li>• Then smoothly and evenly push a second glass slide across the length of the first slide, drawing the liquid in a film over the slide.</li> <li>• Allow samples to air-dry at <math>15</math> to <math>25^\circ\text{C}</math>.</li> </ul>
7	Fix the cells with the Ethanol fixative for at least 20 min at $-15$ to $-25^\circ\text{C}$ .
8	<ul style="list-style-type: none"> <li>• Wash glass slides with cells <math>3 \times</math> with Washing buffer.</li> <li>• Carefully dry the peripheral zone of the area to be stained (e.g., with a cellulose cloth).</li> </ul>
9	<ul style="list-style-type: none"> <li>• Cover the cells with a sufficient amount of Anti-BrdU working solution.</li> <li>• Incubate glass slides for 30 min at <math>37^\circ\text{C}</math> in a humid atmosphere.</li> </ul>
10	<ul style="list-style-type: none"> <li>• Wash glass slides with cells <math>3 \times</math> with Washing buffer.</li> <li>• Carefully dry the peripheral zone of the area to be stained (e.g., with a cellulose cloth).</li> </ul>
11	<ul style="list-style-type: none"> <li>• Cover the cells with a sufficient amount of Anti-mouse-Ig-AP working solution.</li> <li>• Incubate the glass slide for 30 min at <math>37^\circ\text{C}</math> in a humid atmosphere.</li> </ul>
12	<ul style="list-style-type: none"> <li>• Wash glass slides with cells <math>3 \times</math> with Washing buffer</li> <li>• Carefully dry the peripheral zone of the area to be stained (e.g., with a cellulose cloth).</li> </ul>
13	<ul style="list-style-type: none"> <li>• Cover the cells with a sufficient amount of freshly prepared Color-substrate solution</li> <li>• Incubate at <math>15\text{--}25^\circ\text{C}</math> for 15–30 min.</li> </ul>
14	Remove Color-substrate solution carefully by washing with a sufficient amount of Washing buffer and carefully dry the peripheral zone of the area to be stained.
15	Cover the preparations with an appropriate mounting medium (e.g., Kaiser's glycerin-gelatin).
16	Evaluate in a light microscope.

### 3.4 Immunohistochemistry using tissue sections (frozen or paraffin-embedded)

#### 3.4.1 Labeling with BrdU *in vivo*

##### Protocol

In the following protocol the *in vivo* labeling of tissue is described.

Step	Action
1	Inject animal (intravenous) with undiluted BrdU labeling reagent (bottle 1), $1\text{--}2 \text{ ml}/100 \text{ g}$ (body weight).
2	Sacrifice animal 1 h after injection and remove organs to be investigated.
3	Process tissue for frozen sectioning or paraffin-embedding.

#### 3.4.2 Labeling of tissue slices

##### Protocol

In the following protocol the labeling of tissue slices.

Step	Action
1	Place tissue sample in pre-warmed ( $37^\circ\text{C}$ ) cell culture medium.
2	Cut tissue sample with a sharp blade to obtain thin slices (approx. $1 \text{ mm}$ thin and $2 \text{ mm}^2$ in area).
3	<ul style="list-style-type: none"> <li>• Aspirate cell culture medium and add a sufficient amount of BrdU labeling medium.</li> <li>• Incubate for 30–60 min at <math>37^\circ\text{C}</math>, <math>5\% \text{ CO}_2</math>.</li> </ul> <b>Note:</b> The incubation period depends on the tissue type used and the individual requirements.
4	<ul style="list-style-type: none"> <li>• Remove labeling medium and add Washing buffer to the tissue slices.</li> <li>• Incubate for 25 min at <math>37^\circ\text{C}</math>, <math>5\% \text{ CO}_2</math>.</li> </ul>
5	Process tissue slices for frozen sectioning or paraffin-embedding.

#### 3.4.3 Preparation of sections

##### Protocol

The following table describe the preparation of frozen and paraffin-embedded sections.

Preparation of...	Action
frozen sections	<ul style="list-style-type: none"> <li>• Prepare frozen tissue sections in a cryostat (<math>3\text{--}5 \mu\text{m}</math> thick).</li> <li>• Apply sections directly on clean, fat-free, poly-L-lysine coated glass slides (most tissues should be air-dried at <math>+15</math> to <math>+25^\circ\text{C}</math> prior to further use).</li> <li>• Fix sections with the Ethanol fixative for at least 20 min at <math>-15</math> to <math>-25^\circ\text{C}</math>.</li> </ul>
paraffin-embedded sections	<ul style="list-style-type: none"> <li>• Prepare paraffin-embedded sections in a ultramicrotome (<math>3\text{--}5 \mu\text{m}</math> thick).</li> <li>• Take care that the sections are thoroughly dewaxed prior to further use.</li> </ul>

### 3.4.4 Immunohistochemical procedure

#### Protocol

Please refer to the following table:

Step	Action
1	Rehydrate specimen (frozen or paraffin-embedded tissue sections) by washing 3 × with Washing buffer and carefully dry the peripheral zone of the area to be stained (e.g., with a cellulose cloth).
2	<ul style="list-style-type: none"> <li>• Cover the section with a sufficient amount of Anti-BrdU working solution.</li> <li>• Incubate glass slides for 30 min at 37°C in a humid atmosphere.</li> </ul>
3	<ul style="list-style-type: none"> <li>• Wash glass slides 3 × with Washing buffer.</li> <li>• Carefully dry the peripheral zone of the area to be stained (e.g., with a cellulose cloth).</li> </ul>
4	<ul style="list-style-type: none"> <li>• Cover the sections with a sufficient amount of Anti-mouse-Ig-AP working solution.</li> <li>• Incubate the glass slides for 30 min at 37°C in a humid atmosphere.</li> </ul>
5	<ul style="list-style-type: none"> <li>• Wash glass slides 3× with Washing buffer.</li> <li>• Carefully dry the peripheral zone of the area to be stained (e.g., with a cellulose cloth).</li> </ul>
6	<ul style="list-style-type: none"> <li>• Cover the cells with a sufficient amount of freshly prepared color-substrate solution.</li> <li>• Incubate at 15 to 25°C for 15 – 30 min.</li> </ul>
7	<ul style="list-style-type: none"> <li>• Remove Color-substrate solution carefully by washing with a sufficient amount of Washing buffer.</li> <li>• Carefully dry peripheral zone of the area to be stained.</li> </ul>
8	Cover the preparation with an appropriate mounting medium (e.g., Kaiser's glycerin-gelatin).
9	Evaluate in a light microscope.

## 4. Appendix

### 4.1 Changes to Previous Version

- Editorial Changes

### 4.2 References

- 1 Erlanger, B. F. & Beiser, S. M. (1964) *Proc. Natl. Acad. Sci.* **52**, 68–74.
- 2 Gratzner, H. G. (1982) *Science* **218**, 474–475.
- 3 Vanderlaan, M. & Thomas, C. B. (1985) *Cytometry* **6**, 501–505.
- 4 Ellwart, J. & Dörmer, P. (1985) *Cytometry* **6**, 513–520.

### 4.3 Ordering Information

Product	Pack size	Cat. No.
<b>BrdU labeling of proliferating cells</b>		
<b>• In situ assay</b>		
BrdU Labeling and Detection Kit I	1 kit (100 tests)	11 296 736 001
BrdU Labeling and Detection Kit II	1 kit (100 tests)	11 299 964 001
BrdU Labeling and Detection Kit III	1 kit (1000 tests)	11 444 611 001
In Situ Cell Proliferation Kit, FLUOS	1 kit (100 tests)	11 810 740 001
<b>• ELISA</b>		
Cell Proliferation ELISA, BrdU (colorimetric)	1 kit (1000 tests)	11 647 229 001
Cell Proliferation ELISA, BrdU (chemiluminescent)	1 kit (1000 tests)	11 669 915 001
<b>• Single reagents for in situ assays and ELISA applications</b>		
Anti-BrdU, formalin grade	50 µg (500 µl)	11 170 376 001
Anti-BrdU -Fluorescein, formalin grade	50 µg (500 µl)	11 202 693 001
Anti-BrdU -Peroxidase, Fab fragments, formalin grade	15 U	11 585 860 001
FixDenat	4× 100 ml (2000 tests)	11 758 764 001
Measurement of metabolic activity - Quantification in microplate		
Cell Proliferation Kit I (MTT)	1 kit (2500 tests)	11 465 007 001
Cell Proliferation Kit II (XTT)	1 kit (2500 tests)	11 465 015 001
Cell Proliferation Reagent WST-1	2500 tests	11 644 807 001
<b>Additional reagents</b>		
NBT, Solution	3 ml	11 383 213 001
NBT/BCIP Ready-to-Use Tablets	20 tablets	11 697 471 001
NBT/BCIP Stock solution	8 ml	11 681 451 001

\* available from Roche Applied Science

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