5-Bromo-2´-deoxy-uridine Labeling and Detection Kit I

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

Cat. No. 11 296 736 001

Kit for 100 tests

Version 16.0
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Store at −15 to −25°C

1. Preface

1.1 Kit contents

Vial/ Cap	Label	Content including function
1 (red)	BrdU labeling reagent (1000× conc.)	• 1× 10 ml BrdU stock solution (1000 × conc.) in 10 mM phosphate buffered saline (PBS), sterile • for the labeling of DNA
2 (colorless)	Washing buffer concentrate (10× conc.)	• 1× 100 ml PBS (10× conc.) • for wash steps
3 (green)	Incubation buffer	• 1× 100 ml [66 mM Tris buffer, 0.66 mM $MgCl_2$, 1 mM 2-mercaptoethanol] • for the preparation of the BrdU working solution
4 (yellow)	Anti-BrdU with nucleases	• 1× 1 ml anti-BrdU, mouse monoclonal antibody (clone BMG 6H8 IgG1) containing nucleases for DNA denaturation, in PBS/glycerin • binding of in the DNA incorporated BrdU
5 (blue)	Anti-mouse Ig- fluorescein	• from sheep, • immunosorptively purified, lyophilized, stabilized. • binding of the BrdU antibody

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 [^{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. 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

Please refer to the following table.

Stage	Description
1	Cells, tissue explants or organ cultures are incubated 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 µM (4).
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-fluorescein.
5	Bound anti-BrdU monoclonal antibody is visualization by immunofluorescence microscopy.

Advantages

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 I avoids these problems. The antibody preparation contains specific nucleases which allows access to BrdU after fixation in acidic ethanol. Therefore also simultaneous detection of other markers (double staining) is possible.

Application

The kit can be used for the immunofluorescence microscopy detection of BrdU incorporated into cellular DNA.

Sample material

- Cell culture:
  - adherent cells
  - suspension cells
- 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.

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
- BSA, optional
- Ethanol fixative: Add 50 mM glycine solution to 70 ml abs. EtOH to get 100 ml fixative, pH 2.0.
- Mounting medium (*e.g.*, Citifluor).
- Poly-L-lysine coated glass slides, fat-free, use in immunofluorescence of cytocentrifuge, cell smear preparations or frozen sections

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 µM). ***Note***: For *in vivo* labeling undiluted BrdU labeling reagent (1–2 ml/100 g body weight) is needed.	Prepare shortly before use. Store undiluted (1000×) medium in aliquots at −15 to −25°C.	*In vitro* labeling
Anti-BrdU working solution	Dilute anti-BrdU 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-fluorescein stock solution	Dissolve anti-mouse-Ig-fluorescein solution (bottle 5) in 1 ml double dist. water.	Stable at 2–8°C	
Anti-mouse-Ig-fluorescein working solution	Dilute anti-mouse Ig-fluorescein stock solution 1:10 with PBS. If an extended storage is desired, add BSA (bovine serum albumin), 10 mg/ml.	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 2–8°C	Washing purposes

3.2 Immunofluorescence 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_2 for about 15–60 min. ***Note***: 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	• Cover the cells with Anti-BrdU working solution. • Incubate for 30 min at 37°C.
9	Wash the cover slips three times in Washing buffer.
10	• Cover the cells with Anti-mouse-Ig-fluorescein working solution. • Incubate for 30 min at 37°C.
11	Wash the cover slips three times in Washing buffer.
12	Cover the preparations with an appropriate mounting medium (*e.g.,* Citifluor).
13	Examine in a fluorescence microscope. For evaluation by fluorescence microscopy use an excitation wavelength in the range of 450-500 nm (*e.g.*, 488 nm) and detection in the range of 515-565 nm (green).

3.3 Immunofluorescence using suspension cells by cytocentrifuge – or cell smear preparation

Procedure

Please refer to the following table.

Step	Action
1	Centrifuge the cell suspension at 300 × g for 5–10 min and aspirate the supernatant (cell culture medium).
2	Add BrdU labeling medium (0.5 ml/10^6 cells) and resuspend the cells.
3	Incubate the cell suspension for 15–60 min at 37°C, 5% CO_2. ***Note***: The incubation period depends on the cell type and the individual requirements.
4	• Add Washing buffer to the cells. • Spin cell suspension down (5 min, 300 × g). • Remove supernatant carefully.
5	Repeat washing 2 × as described under 4.
6a	Preparing of cytospin-preparations: 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 with a cytocentrifuge.
6b	Preparing of cell smears: • Place 1 drop (approx. 5–10 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 add 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 15–25°C.
7	Fix the cells with the Ethanol fixative for at least 20 min at −15 to −25°C.
8	• Wash glass slides with cells 3× with Washing buffer. • Carefully dry the peripheral zone of the area to be stained (*e.g.,* with a cellulose cloth).
9	• Cover the cells with a sufficient amount of Anti-BrdU working solution. • Incubate glass slides for 30 min at 37°C in a humid atmosphere.
10	• Wash glass slides with cells 3 × with Washing buffer. • Carefully dry the peripheral zone of the area to be stained (*e.g.*, with a cellulose cloth).
11	• Cover the cells with a sufficient amount of Anti-mouse-Ig-fluorescecin working solution. • Incubate the glass slide for 30 min at 37°C in a humid atmosphere.
12	• Wash glass slides with cells 3× with Washing buffer. • Carefully dry the peripheral zone of the area to be stained (*e.g.,* with a cellulose cloth).
13	Cover the preparations with an appropriate mounting medium (*e.g.*, Citifluor).
14	Evaluate in a fluorescence microscope. For evaluation by fluorescence microscopy use an excitation wavelength in the range of 450–500 nm (*e.g.*, 488 nm) and detection in the range of 515–565 nm (green).

3.4 Immunofluorescence 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), 1–2 ml/100 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°C) cell culture medium.
2	Cut tissue sample with a sharp blade to obtain thin slices (approx. 1 mm thin and 2 mm^2 in area).
3	Aspirate cell culture medium and add a sufficient amount of BrdU labeling medium. Incubate for 30–60 min at 37°C, 5% CO_2. ***Note***: 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 min at 37°C, 5% CO_2.
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	Prepare frozen tissue sections in a cryostat (3–5 µm thick). Apply sections directly on clean, fat-free, poly-L-lysine coated glass slides (most tissues should be air-dried at 15-25°C prior to further use). Fix sections with the Ethanol fixative for at least 20 min at −15 to −25°C.
paraffin-embedded sections	Prepare paraffin-embedded sections in a ultramicrotome (3–5 µm thick). Take care that the sections are thoroughly dewaxed prior to further use.

3.4.4 Immunofluorescence procedure

Protocol

Please refer to the following table.

Step	Action
1	Rehydrate specimen (frozen or paraffin-embedded tissue sections) by washing 3 x with Washing buffer and carefully dry the peripheral zone of the area to be stained (*e.g.* with a cellulose cloth).
2	Cover the section with a sufficient amount of Anti-BrdU working solution. Incubate glass slides for 30 min at 37°C in a humid atmosphere.
3	Wash glass slides 3× with Washing buffer. Carefully dry the peripheral zone of the area to be stained (*e.g.* with a cellulose cloth).wie 6
4	Cover the sections with a sufficient amount of Anti-mouse-Ig-fluorescein working solution. Incubate the glass slides for 30 min at 37°C in a humid atmosphere.
5	Wash glass slides 3× with Washing buffer. Carefully dry the peripheral zone of the area to be stained (*e.g.* with a cellulose cloth).wie6
6	Cover the preparation with an appropriate mounting medium (*e.g.* Citifluor).
7	Evaluate in a fluorescence microscope. For evaluation by fluorescence microscopy use an excitation wavelength in the range of 450–500 nm (*e.g.*, 488 nm) and detection in the range of 515– 565 nm (green).

4. Appendix

4.1 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.
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6 RAS (2003) *Biochemica* **3**, 26-28.

4.2 Related products

For a complete overview of related procucts, please visit and bookmark our Cell Biology and Immunochemistry Special Interest Sites at http://www.roche-applied-science.

Product	Pack size	Cat. No.
BrdU labeling of proliferating cells *In situ* assay		
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 (100 tests)	11 444 611 001
In Situ Cell Proliferation Kit, FLUOS	1 kit	11 810 740 001
ELISA		
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
Single reagents for *in situ* assays and ELISA applications		
Anti-BrdU, formalin grade	50 µg (500 µl)	11 170 376 001
Anti-BrdU -Fluorescein, formalin grade	50 µg (500 µ)	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
Measurement of growth related parameters – *In situ* assay		
Anti-Ki-67 (Ki-S5), formalin grade	100 µg	11 742 345 001

* available from Citifluor Ltd., London, Great Britan.

Available printed materials

Guide to Cell Proliferation and Apoptosis Methods	11 675 028 001
LabFAQS	12 115 972 001

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