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

Anti-Bromodeoxyuridine-Peroxidase, Fab fragments
from mouse hybrid cells (clone BMG 6H8)

Version: 07
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Monoclonal antibody to the thymidine-analog 5-bromo-2′-deoxyuridine, formalin grade.
Stabilized, lyophilized

Cat. No. 11 585 860 001 15 U

Store the lyophilizate at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Content
1	Anti-Bromodeoxyuridine-POD, Fab fragments	1 vial

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the lyophilizate is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	Anti-Bromodeoxyuridine-POD, Fab fragments	Store at +2 to +8°C.

Reconstitution

1. Dissolve lyophilizate in 1 ml double-distilled water to a final concentration of 15 U/ml.

2. Store reconstituted antibody conjugate solution for 6 months at +2 to +8°C.

⚠ ***For best results, ensure that the antibody conjugate solution is free of precipitates. If necessary, centrifuge the solution at high speed prior to use. Do not let the preparation dry out during the immunostaining procedure.***

1.3. Additional Equipment and Reagent required

Standard laboratory equipment

- Centrifuge/cytocentrifuge
- Humidified chamber
- Cover slips or chamber slides

For reconstitution of lyophilizate and preparation of solutions

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

- Double-distilled water
- PBS*
- 5-Bromo-2′-deoxyuridine*
- Sterile culture medium
- BSA*

For labeling of suspension cells with BrdU *in vitro*

- Poly-L-lysine-coated slides

For immunostaining

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

- Fixation solution: ethanol/HCl
- ELISA Blocking Reagent* or,
- PBS containing 10% fetal calf serum
- Hemalum (optional)
- POD substrate solution: DAB (diaminobenzidine)

For labeling of adherent and suspension cells with BrdU

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

- Flat-bottom microplates
- Fixation solution: ethanol/HCl
- ELISA Blocking Reagent* or,
- PBS containing 10% fetal calf serum

For immunoassay procedure (ELISA)

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

- POD substrate solution: ABTS*, ABTS Buffer*, TMB*

1.4. Application

Anti-Bromodeoxyuridine-POD can be used for:

- Immunocyto- and immunohistochemistry: qualitative detection of cell proliferation at the single-cell level by light microscopy.
- Immunoassay (ELISA): quantitative measurement of cell proliferation in cell populations.

The antibody POD conjugate allows the direct detection of incorporated BrdU without an additional secondary detection antibody.

2. How to Use this Product

2.1. Before you Begin

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...
10 mM BrdU stock solution, 1,000x conc.	▪ Dissolve 1 g 5-Bromo-2′-deoxyuridine* (MW 307.1) in 325.63 ml PBS*. ▪ Sterilize using a 0.2 to 0.45 µm filter.	Store 5-Bromo-2′-deoxyuridine at +2 to +8°C. ⚠ ***Keep protected from light.*** Store 1,000x BrdU stock solution in aliquots at −15 to −25°C.	Protocols
100 µM BrdU labeling solution, 10x conc.	Dilute the 1,000x BrdU stock solution with sterile culture medium 1:100 to a final concentration of 100 µM BrdU.	Store 10x BrdU labeling solution 1 month at +2 to +8°C. ⚠ ***Keep protected from light.***	Protocols
POD substrate solution	▪ Dissolve 20 mg DAB (diaminobenzidine) and 34 mg imidazole in 50 ml 50 mM Tris/HCl*, pH 7.4. ▪ Shortly before use, add 17 µl H_2O_2 solution, 30% (v/v). ⓘ *The reaction product is brown, and insoluble in ethanol and water.*	⚠ ***Always prepare fresh.***	Immunocyto- and immunohistochemistry.
ABTS*	Dissolve ABTS* substrate powder in ABTS substrate buffer* (1 mg/ml). ⓘ *The reaction product is green and soluble in water.*	–	POD substrate solution for ELISA.
TMB (BM blue*)	Ready to use solution. ⓘ *The reaction product is yellow. Stopping of the substrate reaction with 1 M H_2SO_4 leads to the conversion into a yellow brownish color.*	–	POD substrate solution for ELISA.
Fixation solution	Ethanol/HCl	–	Protocols

2.2. Protocols

Detection of BrdU incorporation by immunocyto- and immunohistochemistry

Labeling of adherent cells with BrdU *in vitro*

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

1. Grow cells on cover slips or on chamber slides to a confluency of approximately 80%.

2. Add 1/10 volume 10x BrdU labeling solution (100 µM) to the culture medium in which the cells are growing.
   – For example, add 10 µl of the BrdU labeling solution to the cells if they were incubated in 100 µl culture medium (final BrdU concentration: 10 µM).

3. Incubate cells for 30 minutes to 4 hours at +37°C in a humidified atmosphere (5% CO_2).
   – The incubation time in the presence of BrdU (labeling period) depends on the cell type used and the individual experimental requirements.

4. Remove the culture medium containing BrdU and wash the cover slips or chamber slides 3 times with PBS.

5. For cell fixation and immunostaining, see section, **Immunostaining**.

Labeling of suspension cells with BrdU *in vitro*

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

1. Adjust cell concentration to approximately 2 to 5 × 10^5 cells/ml.

2. Add 1/10 volume 10x BrdU labeling solution (100 µM) to the culture medium in which the cells are incubated (final BrdU concentration: 10 µM).

3. Incubate cells for 30 minutes to 4 hours at +37°C in a humidified atmosphere (5% CO_2).
   – The incubation time in the presence of BrdU (labeling period) depends on the cell type used and the individual experimental requirements.

4. Centrifuge cells tor 5 minutes at 300 × *g*.
   – Remove the supernatant carefully.

5. Resuspend the cell pellet in fresh culture medium.

6. Centrifuge the cell suspension for 5 minutes at 300 × *g*; repeat this washing step.

7. **For the preparation of cytospins**, resuspend the cells in culture medium to obtain a concentration of approximately 3 × 10^5 cells/ml.
   – Centrifuge 100 µl of this cell suspension onto a clean poly L-lysine-coated glass slide using a cytocentrifuge.
   – Air-dry the samples at +15 to +25°C.

8. **For the preparation of cell smears**, resuspend the cells in culture medium to obtain a concentration of approximately 5 × 10^7 cells/ml.
   – Place 1 drop of this cell suspension on one end of a clean, poly L-lysine-coated glass slide.
   – To obtain a cell smear, spread the liquid over the glass slide using a second clean slide.
   – Air-dry the samples at +15 to +25°C.

9. For cell fixation and immunostaining, see section, **Immunostaining**.

Labeling of the cells with BrdU *in vivo*

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

1. Inject the animal intraperitoneally with the undiluted 1,000x BrdU stock solution (10 mM).
   1 ml of the BrdU stock solution per 100 g body weight is suitable for most applications.

2. Sacrifice the animal approximately 1 to 2 hours later and remove the tissue or organ under study.

3. Process tissue samples for frozen sectioning or paraffin embedding following standard protocols.
   – For formalin-fixed, paraffin-embedded sections, dewax sections prior to proceeding.

4. Shortly before immunostaining, incubate the sections with POD blocking solution 1 or POD blocking solution 2 for 20 minutes at +15 to +25°C to block endogenous POD activity.

5. Wash the slides 3 times with PBS.

6. For cell fixation and immunostaining, see section, **Immunostaining**.

Immunostaining

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

1. Fix the sample material (frozen sections, dewaxed paraffin sections, cells grown on slides or cover slips, cytospin or cell smear preparations) with fixation solution (ethanol/HCl) for 10 minutes at +15 to +25°C.

2. Wash the slides or cover slips 3 times in PBS containing 1% ELISA Blocking Reagent* or PBS containing 10% fetal calf serum.

3. Cover the preparation with a suitable volume of Anti-BrdU-POD solution (0.5 to 1.0 U/ml).
   – Incubate for 30 minutes at +15 to +25°C in a humidified chamber.

4. Wash the slides or cover slips 3 times with PBS.
   – Wipe slides dry except the area of the preparation after the last washing step.

5. Cover the slides or cover slips with freshly prepared substrate solution and incubate at +15 to +25°C until a clearly visible color develops.
   A negative control should not show any color change during this incubation period.

6. Rinse off substrate solution with PBS.
   – If necessary, counterstain cells with hemalum and mount the preparations.

Detection of BrdU incorporation by immunoassay (ELISA)

Labeling of adherent cells with BrdU

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

1. Culture cells to 80% confluency in 96-well, flat-bottom microplates.

2. Add 1/10 volume of 10x BrdU labeling solution (100 µM) to the culture medium in which the cells are growing to obtain a final concentration of 10 µM BrdU.
   – For example, add 10 µl/well BrdU labeling solution to cells cultured in 100 µl/well culture medium.

3. Incubate cells for 1 to 8 hours or overnight at +37°C in a humidified atmosphere (5% CO_2).
   – The incubation time in the presence of BrdU (labeling period) depends on the cell type used and the individual experimental requirements.

4. Remove culture medium containing BrdU.

5. Gently wash cells once with PBS.

6. Fix cells by adding 100 µl/well fixation solution.
   – Incubate for 20 minutes at +15 to +25°C.

7. Remove fixation solution and wash cells 3 times with PBS containing 1% ELISA Blocking Reagent* or PBS containing 10% fetal calf serum.

8. For detection of incorporated BrdU, see section, **Immunoassay procedure (ELISA)**.

Labeling of suspension cells with BrdU

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

1. Culture cells at a density of 1 to 5 × 10^4 cells/well in a flat-bottom microplate.

2. Add 1/10 volume of 10x BrdU labeling solution (100 µM) to the culture medium in which the cells are growing to obtain a final concentration of 10 µM BrdU.
   – For example, add 10 µl/well BrdU labeling solution if they were cultured in 100 µl/well culture medium.

3. Incubate cells 1 to 8 hours or overnight at +37°C in a humidified atmosphere (5% CO_2).
   – The incubation time in the presence of BrdU (labeling period) depends on the cell type used and the individual experimental requirements.

4. Centrifuge cells for 10 minutes at 300 × *g*.
   – Remove the supernatant carefully by aspiration using a cannula.
   ⚠ ***Do not shake the cells settled on the bottom of the culture plate.***

5. Dry cells at +60°C for approximately 2 hours.

6. Add 100 µl/well fixation solution.
   – Incubate for 20 minutes at +15 to +25°C.

7. Remove fixation solution and wash fixed cells 3 times with PBS containing 1% ELISA Blocking Reagent* or PBS containing 10% fetal calf serum.

8. For detection of incorporated BrdU, see section, **Immunoassay procedure (ELISA)**.

Immunoassay procedure (ELISA)

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

1. Add 100 µl/well Anti-BrdU-POD solution (75 mU) to the fixed and washed cells.
2. Incubate for 30 minutes at +15 to +25°C.
3. Wash the microplate 3 times with PBS.
4. Add 100 µl/well freshly prepared POD substrate solution.
5. Incubate for 5 to 30 minutes at +15 to +25°C.
   When using TMB (tetramethylbenzidine) as substrate, the reaction can be stopped by adding 50 µl/well 1 M H_2SO_4.*
6. Measure absorbance of the samples in an ELISA reader at the following wavelengths:

Substrate	Wavelength [nm]
ABTS	405
TMB (BM blue) (unstopped)	370
TMB (BM blue) (stopped)	450

2.3. Parameters

Specificity

- The antibody reacts with the thymidine analog BrdU and with BrdU incorporated into DNA. For binding to BrdU incorporated into the DNA, the BrdU-labeled DNA must be denatured/fixed and/or partially degraded to obtain single-stranded DNA.
- The antibody does not cross-react with any endogenous cellular components such as thymidine, uridine, or DNA.

Working Concentration

Application	Concentration [U/ml]
Immunohistocytochemistry	0.5 – 1.0
ELISA immunoassay	0.20

Dilute the antibody conjugate shortly before use with phosphate buffered saline (PBS) containing 1% BSA* (w/v).*

3. Additional Information on this Product

3.1. Test Principle

During apoptosis, caspases cleave intracellular proteins; the corresponding caspase cleaving sites are formalin resistant. Formalin-grade antibodies recognize cleavage sites in apoptotic cells that are not accessible in normal cells, which allows for determination of caspase activity, even in formalin-fixed, paraffin-embedded tissue.
Bromodeoxyuridine (BrdU) is a thymidine analog and is specifically incorporated by proliferating cells into cellular DNA during DNA synthesis (= S phase of the cell cycle).

- This process is analogous to the long-established ^{3}H thymidine uptake technique, but eliminates the need for radioactivity.
- An antibody directed against BrdU allows the identification of cells which have already incorporated BrdU.
- These antibodies label only those cells that have incorporated BrdU during DNA replication.

Preparation

1. BALB/c mice were immunized 3 times with a bromodeoxyuridine coupled to bovine serum albumin (BSA).
2. The spleen cells were isolated and fused with P3-X63-Ag8.653 myeloma cells.
3. Hybridoma supernatants were screened for binding to the immunogen and for nonbinding to BSA and non-BrdU-substituted cellular DNA, respectively.
4. Hybridomas producing specific monoclonal antibodies against BrdU were selected and cloned by limiting dilution.
5. The antibody was purified by ion exchange chromatography, degraded to Fab fragments enzymatically, and conjugated with peroxidase (POD).
6. The anti-BrdU-POD Fab fragments were diluted in 50 mM HEPES, pH 7.4, 8% saccharose, (w/v), 2% mannit (w/v), 25 µM $CaCl_2$, 0.4% BSA* (w/v), 10 mM potassium hexacyanoferrate II, 0.01% 2-methylisothiazolone (MIT) (w/v), and lyophilized.

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

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
5-Bromo-2'-deoxyuridine	1 g	10 280 879 001
Tris hydrochloride	500 g	10 812 846 001
Buffers in a Box, Premixed PBS Buffer, 10x	4 l	11 666 789 001
ABTS	2 g	10 102 946 001
Blocking Reagent	27 g, for one liter blocking solution, *Not available in US*	11 112 589 001
ABTS Buffer	ABTS Buffer, 125 ml	11 204 530 001
BM Blue POD Substrate, soluble	100 ml	11 484 281 001
Bovine Serum Albumin Fraction V	50 g	10 735 078 001
	100 g, *Not available in US*	10 735 086 001
	500 g, *Not available in US*	10 735 094 001
	1 kg, *Not available in US*	10 735 108 001

4.4. Trademarks

ABTS is a trademark of Roche.
All other 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.

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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