## Overview

<table>
<thead>
<tr>
<th><strong>Product name</strong></th>
<th>Anti-BrdU antibody [BU1/75 (ICR1)]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Description</strong></td>
<td>Rat monoclonal [BU1/75 (ICR1)] to BrdU</td>
</tr>
<tr>
<td><strong>Host species</strong></td>
<td>Rat</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>This antibody reacts with BrdU (5-Bromo-2'-deoxyuridine) in single stranded DNA, BrdU attached to a protein carrier or free BrdU. It detects nucleated cells that have had BrdU incorporated into their DNA during S phase. The antibody clone has been reported to react with EdU (5-Ethynyl-2'-deoxyuridine, see PMID: 23272138 DOI: 10.1371/journal.pone.0051679). It reacts with CldU (5-Chloro-2'-deoxyuridine) but with reduced staining. Some customers have found that this antibody also reacts with IdU (5-Iodo-2'-deoxyuridine).</td>
</tr>
<tr>
<td><strong>Tested applications</strong></td>
<td><strong>Suitable for:</strong> ICC/IF, IHC-FoFr, IHC-P, IHC-Fr, Flow Cyt, IHC-FrFl</td>
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<tr>
<td><strong>Species reactivity</strong></td>
<td>Not applicable.</td>
</tr>
<tr>
<td><strong>Immunogen</strong></td>
<td>The details of the immunogen for this antibody are not available.</td>
</tr>
</tbody>
</table>
| **General notes** | **Protocol advice:**
This antibody recognizes single stranded DNA so the DNA needs to be unraveled first. This can be done with DNAse, although it doesn't give the best results. Depending on the assay, acid denaturation with 2M HCL or heat denaturation are the most successful. Please note this step is critical in any assay with this antibody and is the area that should be modified to optimize results. A detailed BrdU staining protocol is available in the Protocols tab or by clicking on this link.
This monoclonal antibody is manufactured exclusively by Abcam.
AF488 conjugate available as ab220074
AF647 conjugate available as ab220075
Please see the Associated Products tab for other available conjugates.
If you require this antibody in a particular buffer formulation or a particular conjugate for your experiments, please contact orders@abcam.com or you can find further information here. |

## Properties

<table>
<thead>
<tr>
<th><strong>Form</strong></th>
<th>Liquid</th>
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<tbody>
<tr>
<td><strong>Storage instructions</strong></td>
<td>Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.</td>
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</tbody>
</table>
| **Storage buffer** | pH: 7.4
Preservative: 0.02% Sodium azide |
Constituent: PBS

Some batches contain 6.97% L-Arginine as a stabilizing agent. For lot-specific buffer information, please contact our Scientific Support team.

**Purity**
- IgG fraction

**Clonality**
- Monoclonal

**Clone number**
- BU1/75 (ICR1)

**Isotype**
- IgG2a

**Light chain type**
- kappa

### Applications

Our **Abpromise guarantee** covers the use of **ab6326** in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>ICC/IF</td>
<td>★★★★★</td>
<td>1/250.</td>
</tr>
<tr>
<td>IHC-FoFr</td>
<td>★★★★★</td>
<td>1/40.</td>
</tr>
<tr>
<td>IHC-P</td>
<td>★★★★★</td>
<td>1/25 - 1/200.</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td>★★★★★</td>
<td>1/40 - 1/200. PubMed: 16670699; In addition, found to work at 1/400. For PFA fixed tissue use at 1/40, from PMID 16373695.</td>
</tr>
<tr>
<td>Flow Cyt</td>
<td>★★★★★</td>
<td>1/25 - 1/200. Rat IgG2a, kappa monoclonal [RTK2758] (Low endotoxin, Azide Free) (ab18450), is suitable for use as an isotype control with this antibody.</td>
</tr>
<tr>
<td>IHC-FrFl</td>
<td>★★★★★</td>
<td>Use at an assay dependent concentration.</td>
</tr>
</tbody>
</table>

### Target

**Relevance**

The immunocytochemical detection of bromodeoxyuridine (BrdU) incorporated into DNA is a powerful tool to study the cytokinetics of normal and neoplastic cells. In vitro or in vivo labeling of tumor cells with the thymidine analogue BrdU and the subsequent detection of incorporated BrdU with specific anti-BrdU monoclonal antibodies is an accurate and comprehensive method to quantitate the degree of DNA-synthesis. BrdU is incorporated into the newly synthesized DNA of S-phase cells may provide an estimate for the fraction of cells in S-phase. Also dynamic proliferative information such as the S-phase transit rate and the potential doubling time can be obtained, by means of bivariate BrdU/DNA flow cytometric analysis.

**Cellular localization**
- Nuclear

### Images
Immunocytochemistry / Immunofluorescence - Anti-BrdU antibody [BU1/75 (ICR1)] (ab6326)

ab6326 stained in Hela cells. Untreated and BrdU treated (10uM for 24 hours) cells were fixed with 100% methanol (5 min) and then subjected to acid hydrolysis using 2M HCL in 0.1% PBS-Tween for 30 minutes at room temperature to denature the DNA. They were then incubated with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% triton for 1h at room temperature to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab6326 at 5µg/ml and ab7291 (Mouse monoclonal to alpha tubulin) at 1ug/ml overnight at +4°C. The secondary antibodies were Goat Anti-Rat IgG H&L (Alexa Fluor® 488) preadsorbed (ab150165) (colored green) used at 2 ug/ml and Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed (ab150120) (pseudocolored red) used at 1/1000 dilution for 1hour at room temperature. DAPI was used to stain the cell nuclei (colored blue) at a concentration of 1.43µM for 1hour at room temperature.

(A) Schematic of BrdU pulse labeling. (B) E15.5 whole brain section incorporated with BrdU after IUE at E13.5. Dashed rectangle indicates cortical area for quantification. Scale bar, 200 μm. (C) BrdU incorporation in mice subjected to IUE with indicated plasmids. Scale bar, 50 μm.

Mouse brain slices were washed in PBS for 3 times and permeated in 0.3% (v/v) Triton X-100 in PBS for 30 min at room temperature (RT). After treatments, brain sections were incubated directly in a blocking solution (10% (v/v) donkey serum in PBS) for 1 hr, followed by the incubation with the primary antibodies at 4°C overnight. Sections were then washed with PBS for 3 times followed by incubation with the appropriate secondary antibodies for 1–2 hr at room temperature (RT). Sections were mounted with fluorescent mounting medium and stored at 4°C. ab6326 was used at a dilution of 1:1,000. Images were acquired on a Nikon A1R laser confocal microscope except that the labeled brain slices from time-lapse imaging were imaged on the Olympus FV10i-O with a 10x (zoom x2) air objective lens.
Confocal immunofluorescence sections (~0.3 μm thick) showing the localization of the different factors in HEK 293 cells infected with Ad5 wt. Double labeling for BrdU (ab6326, magenta) and L1 52/55 kDa (green). Dotted rectangles indicate the areas shown at higher magnification in the bottom row. Bars: 3 μm.

Monolayer HEK293 cells grown in cover glasses were infected with Ad5 wt. The infection was synchronized by incubating the cells for 30 min at 4°C and then 30 min at 37°C. Then, the inoculums were removed and medium was added. For BrdU labeling, after 18 h at 37°C the medium was changed by medium containing 25 μg/ml BrdU (5-Bromo-2'-deoxyuridine), followed by another change at 25 hpi. Incubation with BrdU proceeded at 37°C. After 36 hpi, the medium was removed and 4% paraformaldehyde in PBS was added to the cells during 10 min. After 3 rinses with PBS, cover glasses were incubated with a mixture of 0.5% saponin and 10% FBS in PBS for 10 min. Samples were incubated with the primary antibody in 0.5% saponin and 2% FBS in PBS during 45 min. After three more rinses, incubation with secondary antibodies diluted in 0.5% saponin and 2% FBS in PBS was carried out in darkness. After a final rinse with PBS, cover glasses were mounted on glass slides. Antifade reagent was allowed to dry overnight before sample observation. All incubations were carried out at room temperature.

Images were taken using a confocal multispectral Leica TCS SP5 system.

SPF mice (4-week-old) were provided with drinking water supplemented (or not) with an antibiotic cocktail (Abx: ampicillin, vancomycin, metronidazole, neomycin) for 4 weeks, after which frozen sections of the ileum from mice (8-week-old) were prepared at 2 h or 2, 3, or 5 days after BrdU injection and were immunostained with mAbs to BrdU (red) and to β-catenin (green). The boxed areas in the left panels for the sections prepared at 2 h after BrdU injection are shown at higher magnification in the right panels. Scale bars, 20 μm (higher magnification) or 100 μm (lower magnification).

Mice were injected intraperitoneally with BrdU (10 mg per kilogram of body weight). After 2, 48, 72, or 120 h, the ileum was removed and fixed with 4% paraformaldehyde, transferred to a series of sucrose solutions in PBS, embedded in OCT compound, and rapidly frozen with liquid nitrogen. Sections with a thickness of 5 μm were incubated for 30 min at 65°C with 0.025 M HCl, washed with 0.1 M borate buffer (pH 8.5), and incubated at room temperature first for 2 h with ab6326 and anti-β-catenin and then for 1 h with fluorescent dye–labeled secondary antibodies. Fluorescence
images were obtained with a fluorescence microscope (BX51, Olympus).

Dot plot showing BrdU-treated HeLa cells stained with ab6326. Cells were incubated with 10 µM BrdU for 30 minutes prior to being harvested, washed twice in 1x PBS and fixed in 70% ethanol (4°C, added drop-wise) for at least 30 minutes on ice. Once fixed, pellets were acid denatured with 2M HCl for 30 minutes at 22°C and then neutralised with borate buffer (0.1M, pH8.5).

Samples were washed and incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab6326, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was Goat Anti-Rat IgG H&L (Alexa Fluor® 488) preadsorbed (ab150165) at 1/2000 dilution for 30 min at 22°C.

7-AAD was added to cells 20 min prior to data acquisition.

Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) with 530/30 and 685/35 bandpass filters.

ICC/IF image of ab6326 stained HeLa cells, both BrdU treated (left image) and normal cells (right image). The cells were 100% methanol fixed (5 min) and then subjected to acid hydrolysis using 2M HCL in 0.1% PBS-Tween for 30 minutes at room temperature to denature the DNA. They were then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab6326, 10µg/ml) overnight at +4°C. The secondary antibody (green) was Goat Anti-Rat IgG H&L (DyLight® 488) preadsorbed (ab98420) used at a 1/250 dilution for 1h. Alexa Fluor® S94 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM. Positive staining can be seen in the BrdU treated cells, but not in the normal cells, demonstrating specificity for BrdU.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-BrdU antibody [BU1/75 (ICR1)] (ab6326)

IHC image of ab6326 staining in a formalin-fixed, paraffin-embedded rat small intestine BrdU tissue section. The section was pre-treated using pressure cooker heat mediated antigen retrieval with sodium citrate buffer (pH6) for 30mins, and incubated overnight at +4°C with ab6326 at 1 ug/ml. A goat anti-rat biotinylated secondary antibody was used to detect the primary, and visualized using an HRP conjugated ABC system. The section was counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

Immunohistochemistry (PFA perfusion fixed frozen sections) - Anti-BrdU antibody [BU1/75 (ICR1)] (ab6326)

This image is courtesy of an Abreview submitted by Musaad Atrashmari.

ab6326 staining BrdU in mouse brain (dentate gyrus) tissue sections by Immunohistochemistry (PFA perfusion fixed frozen sections). Tissue samples were perfused with 1X PBS followed by 4% paraformaldehyde and then cryopreserved in 20-30% sucrose. 20-25 µm sections were permeablized with 1% Triton X-100 + 0.5% Tween 20 in 1X PBS. Sections were treated with 1 N HCL for 10 min followed by 2 N HCL for 10 min at RT and then 20 min at 37°C. Then sections were incubated with borate for pH correction and permeabized with 1 X TBS and blocked with 3-5% donkey serum. Samples were incubated with the primary antibody (1/1000 in 1X PBS + 0.1% Tween 20) at 4°C overnight. Donkey Anti-Rat IgG H&L (Alexa Fluor® 568) preadsorbed (ab175475) (1/250) was used as the secondary antibody.

Green - DCX.
Red - BrdU.
Blue - NeuN.
Immunohistochemistry (Frozen sections) - Anti-BrdU antibody [BU1/75 (ICR1)] (ab6326)
This image is courtesy of an anonymous Abreview

ab6326 staining BrdU in mouse brain tissue sections by IHC-Fr (paraformaldehyde-fixed frozen sections). Tissue samples were fixed with paraformaldehyde; permeabilized with 0.3% Triton X-100 and blocked with 5% Serum for 2 hours at 4°C. Before permeabilization samples were pretreated with 2N HCl at RT for 30 min and washed 3 times. The sample was incubated with primary antibody (1/200) at 4°C for 12 hours. An Alexa Fluor® 488-conjugated Goat polyclonal to rat IgG (1/500) was used as secondary antibody. BrdU staining shown in green and NeuN staining shown in red.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-BrdU antibody [BU1/75 (ICR1)] (ab6326)
This image is courtesy of an anonymous Abreview

ab6326 staining cultured cells of rat brain tissue by ICC. The sample was PFA fixed and permeabilized in 1M HCl prior to blocking with 5% serum for 1 hour at 25°C. The primary antibody was diluted 1/500 and incubated with the sample for 16 hours at 25°C. A biotinylated rabbit anti-rat IgG antibody, diluted 1/200, was used as the secondary.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-BrdU antibody [BU1/75 (ICR1)] (ab6326)


Immunohistological staining of frontal (A-C) and sagittal (D) sections through a tooth family on the lower jaw of *S. salar* with BrdU labelled cells in green and DAPI counterstain in blue. The tooth families presented are in a similar state of development: a young functional tooth and its successor in morphogenesis stage. (A) Pulsed specimen, T0; (B) Chase time T1, one week after BrdU administration; (C) Chase time T2, two weeks after BrdU administration and (D) Chase time T3, four weeks after BrdU administration. The white arrow indicates single cells within the middle dental epithelium (MDE) enclosed by the IDE, cervical loop and ODE of the replacement tooth. Abbreviations: Ab: aboral; Ant: anterior; CV: cervical loop; DP: dental papilla; FT: functional tooth; IDE: inner dental epithelium; Lab: labial; Lin: lingual; MDE: middle dental epithelium; ME: mesenchyme; OE: oral epithelium; ODE: outer dental epithelium; Or: oral; PC: pulp cavity; Pos: posterior; yellow asterisk: replacement tooth; scale bars: 100 μm.

Immunohistological staining for BrdU on the paraffin sections:
Rehydration through a decreasing ethanol series, chromatin precipitation in hydrochloric acid, block in 3% BSA/1% milk powder, exposure to primary antibody (ab6326) and secondary antibody (polyclonal anti-rat Alexa Fluor 488), DAPI counterstaining (1μl/ml). Immunofluorescence was visualized on a NIKON eclipse TE2000-S confocal laser-scanning microscope. Adobe Illustrator CS5, Adobe Photoshop CS5 and Fiji were used to process bright field and fluorescent images of the immunostained sections.

Thoracotomy triggers cell proliferation in the intact zebrafish heart. (c,d) Representative sections of hearts after one week of BrdU (green) treatment. Mef2 staining (red) was performed to differentiate CM nuclei from non-CM nuclei. (c’,d’,c”,d”) Higher magnifications of the framed area shown in the images that are labelled with the same letter without the prime symbol. Arrows indicate double-positive nuclei.

Hearts were collected and fixed overnight at 4°C in 2% paraformaldehyde. They were then rinsed in PBS and equilibrated in 30% sucrose before embedding in OCT compound and cryosectioned at a thickness of 16 µm. The slides were incubated in 2 M HCl in PBS with 0.3% Triton-X for 45 min before the immunohistochemistry procedure. Rat anti-BrdU was used at a 1/100 dilution (ab6326). An Alexa-Fluor-conjugated secondary antibody was used at 1/500 and DAPI was used at 1/2000. Counterstained with DAPI.

Immunohistochemistry (Frozen sections) - Anti-BrdU antibody [BU1/75 (ICR1)] (ab6326)

ab6326 staining BrdU in HeLa cells by Flow Cytometry. Cells were incubated with 10 µM BrdU for 30 minutes prior to being harvested with 1X trypsin-EDTA, washed twice in PBS containing 1% BSA, and fixed in 70% ethanol (added drop-wise) for at least 30 minutes on ice. Once fixed, pellets were acid denatured with HCl/Triton X-100 for 30 minutes at room temperature and then neutralised with sodium tetraborate.

Pelleted cells were re-suspended in Tween/BSA/PBS to which primary antibody was then added (0.1 µg in 0.5% Tween 20 (v/v) plus 1% BSA in PBSA) and incubated for 30 minutes at room temperature. Secondary Alexa Fluor®488-conjugated Goat anti-Rat IgG (H+L) was used at 1/500 and incubated for 30 minutes at room temperature in the dark. Cells were pelleted once more and resuspended in PBS containing 5 µg/mL propidium iodide.

**Gating Strategy:** Based on forward and side scatter, cells were gated into the region used for analysis. This was done by applying a large circle to a

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**Please note:** All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

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