abcam

Product datasheet

Anti-BrdU antibody [BU1/75 (ICR1)] - Proliferation Marker ab6326

104 Abreviews 1734 References 9 Images

Overview

Product name	Anti-BrdU antibody [BU1/75 (ICR1)] - Proliferation Marker
Description	Rat monoclonal [BU1/75 (ICR1)] to BrdU - Proliferation Marker
Host species	Rat
Specificity	This antibody reacts with BrdU in single stranded DNA, BrdU attached to a protein carrier or free BrdU. It detects nucleated cells in S-Phase which have had BrdU incorporated into their DNA. Also reacts with chlorodeoxyuridine but with reduced staining. The antibody does not react with thymidine. It has been reported in the literature that this antibody clone cross-reacts with Edu (PMID: 23272138) and some customers reported that it cross reacts with IdU.
Tested applications	**Suitable for:** ICC/IF, Flow Cyt (Intra), IHC-P
Species reactivity	**Reacts with:** Species independent
Immunogen	The details of the immunogen for this antibody are not available.
Positive control	ICC/IF: HeLa cells; Flow Cyt (Intra): HeLa cells; IHC-P: Rat small intestine tissue.
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**. Unstained positive control slides from mice treated with BrdU (formalin-fixed, paraffin-embedded intestine sections) are available as BrdU control slides **ab129956**. 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**. The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	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.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine Some batches contain 6.97% L-Arginine as a stabilizing agent. For lot-specific buffer information, please contact our Scientific Support team.
Purity	Protein G purified
Clonality	Monoclonal
Clone number	BU1/75 (ICR1)
Isotype	IgG2a
Light chain type	kappa

Applications

The Abpromise guarantee 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.

Application	Abreviews	Notes
ICC/IF	★★★★★ **(26)**	1/250.
Flow Cyt (Intra)		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.
IHC-P	★★★★★ **(28)**	Use a concentration of 1 - 3 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

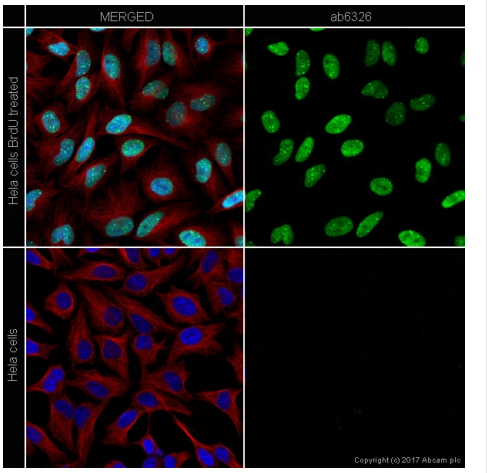
Target

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 synthezised 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.

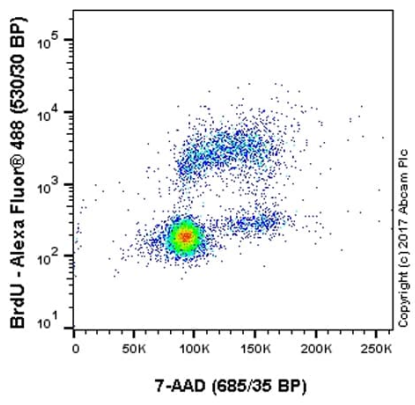
Cellular localization Nuclear

Images



Immunocytochemistry/ Immunofluorescence - Anti-BrdU antibody [BU1/75 (ICR1)] - Proliferation Marker (ab6326)

ab6326 staining BrdU in Hela cells. Untreated and BrdU treated (10uM for 24 hours) cells. The 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 permeabilized with 0.1% PBS-Tween for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1hr. The cells were then incubated overnight at 4°C with ab6326 at 1µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with **ab150165**, Goat polyclonal Secondary Antibody to Rat IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue). Image was acquired with a confocal microscope (Leica-Microsystems TCS SP8) and a single confocal section is shown.



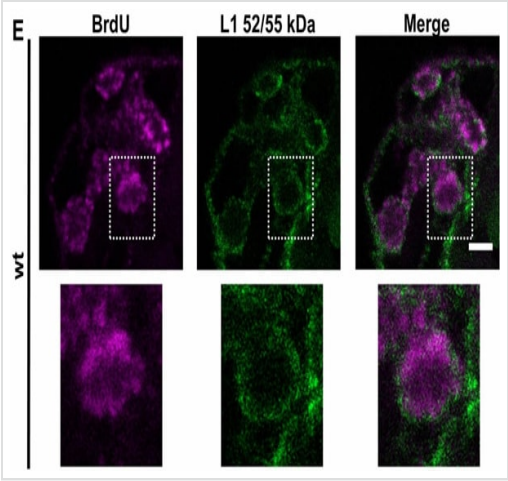
Flow Cytometry (Intracellular) - Anti-BrdU antibody [BU1/75 (ICR1)] - Proliferation Marker (ab6326)

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 22ºC 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 22ºC.

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

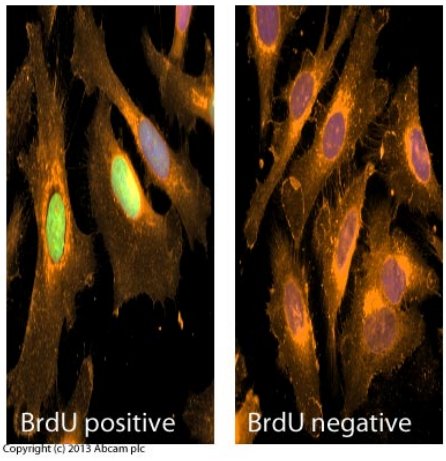
Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) with 530/30 and 685/35 bandpass filters.



Immunocytochemistry/ Immunofluorescence - Anti-BrdU antibody [BU1/75 (ICR1)] - Proliferation Marker (ab6326)

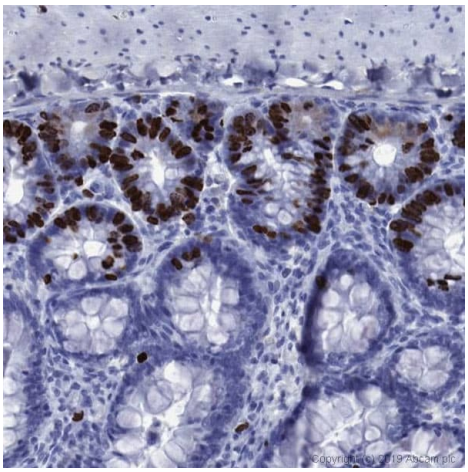
Confocal immunofluorescence sections (~0.3 µm thick) showing the localization of the different factors in HEK 293 cells infected with Ad5 wt. Double labeling for BrdU (ab6326, magenta) and L1 52/55 kDa (green). Dotted rectangles indicate the areas shown at higher magnification in the bottom row. Bars: 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.



Immunocytochemistry/ Immunofluorescence - Anti-BrdU antibody [BU1/75 (ICR1)] - Proliferation Marker (ab6326)

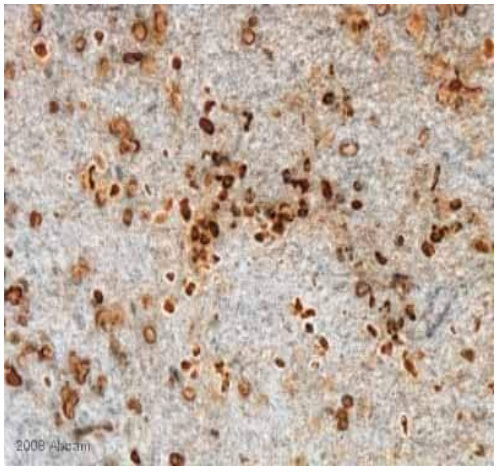
ICC/IF image of ab6326 stained HeLa cells, both BrdU treated (left image) and normal cells (right image). The cells were 100% methanol fixed (5 min) and then subjected to acid hydrolysis using 2M HCL in 0.1% PBS-Tween for 30 minutes at room temperature to denature the DNA. They were then incubated in 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® 594 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)] - Proliferation Marker (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 3 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.

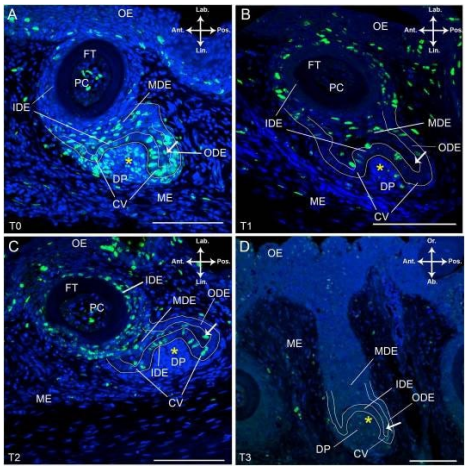
For other IHC staining systems (automated and non-automated) users should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-BrdU antibody [BU1/75 (ICR1)] - Proliferation Marker (ab6326)

This image is courtesy of an anonymous Abreview

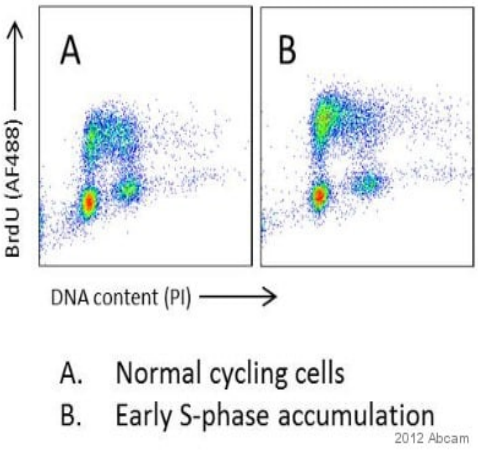
ab6326 staining cultured cells of rat brain tissue by ICC. The sample was PFA fixed and permeabilized in 1M HCl prior to blocking with 5% serum for 1 hour at 25°C. The primary antibody was diluted 1/500 and incubated with the sample for 16 hours at 25°C. A biotinylated rabbit anti-rat IgG antibody, diluted 1/200, was used as the secondary.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-BrdU antibody [BU1/75 (ICR1)] - Proliferation Marker (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 eclips 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.

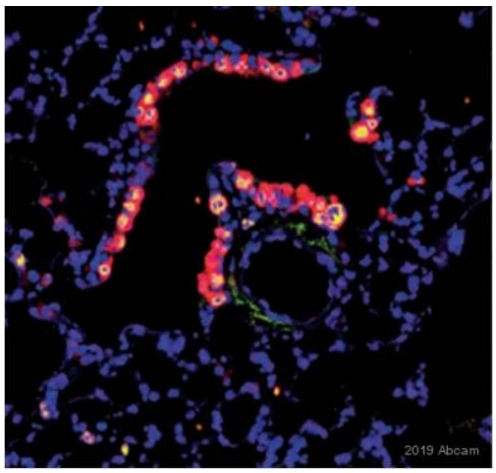


Flow Cytometry (Intracellular) - Anti-BrdU antibody [BU1/75 (ICR1)] - Proliferation Marker (ab6326)

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



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

ab6326 staining BrdU in mouse lung tissue sections by Immunohistochemistry (Formalin/PFA-fixed parafin-embedded sections). Tissue samples were heat mediated with Citrate buffer for 40min. The sections were blocked with 10% donkey serum for 1 hour at 22°C. Samples were incubated with the primary antibody (1/200 in 10% Donkey serum-PBS 0.2% Triton) at 4°C for 16 hours. Cy™3 AffiniPure Donkey Anti-Rat IgG (H+L) (1/400) was used as the secondary antibody.

Representative confocal images of terminal bronchioles in mouse lungs taken at day 5 following naphthalene injection.

Colors label CC10 (red), FoxJ1 (green), BrdU (yellow), and nuclear DNA (blue).

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