

Datasheet: MCA2060GA

Description:	RAT ANTI BrdU
Specificity:	BrdU
Other names:	5-BROMODEOXYURIDINE
Format:	Purified
Product Type:	Monoclonal Antibody
Clone:	BU1/75 (ICR1)
Isotype:	IgG2a
Quantity:	0.1 mg

Product Details

Applications

This product has been reported to work in the following applications. This information is derived from testing within our laboratories, peer-reviewed publications or personal communications from the originators. Please refer to references indicated for further information. For general protocol recommendations, please visit www.bio-rad-antibodies.com/protocols.

	Yes	No	Not Determined	Suggested Dilution
Flow Cytometry (1)	■			1/25 - 1/200
Immunohistology - Frozen			■	
Immunohistology - Paraffin (2)	■			1/25 - 1/200
ELISA			■	
Immunoprecipitation			■	
Western Blotting			■	
Immunofluorescence	■			

Where this antibody has not been tested for use in a particular technique this does not necessarily exclude its use in such procedures. Suggested working dilutions are given as a guide only. It is recommended that the user titrates the antibody for use in their own system using appropriate negative/positive controls.

(1) See recommended protocol below.

(2) See recommended protocol below.

Target Species	Chemical
Product Form	Purified IgG - liquid
Preparation	Purified IgG prepared by affinity chromatography on Protein G from tissue culture supernatant
Buffer Solution	Phosphate buffered saline
Preservative Stabilisers	0.09% Sodium Azide (NaN ₃)
Approx. Protein Concentrations	IgG concentration 1.0mg/ml

Specificity	Rat anti BrdU antibody clone BU1/75 (ICR1), recognizes bromodeoxyuridine (known as BrdU or BrdUrd). Rat anti BrdU antibody clone BU1/75 (ICR1) reacts with BrdU incorporated into single stranded DNA, attached to a protein carrier and free BrdU. Rat anti BrdU antibody, clone BU1/75 (ICR1) cross reacts with chlorodeoxyuridine (CldU) but does not cross react with thymidine or iododeoxyuridine (Aten et al. 1992). BrdU, IdU and CldU are analogs of thymidine, they can incorporate into DNA during DNA synthesis replacing thymidine. Antibody detection of incorporated BrdU in cellular DNA is extensively referenced as an accurate method to monitor cell proliferation <i>in vivo</i> and <i>in vitro</i>. In cell proliferation assays BrdU staining is coupled with the use of a dye that binds total DNA such as propidium iodide (PI). BrdU can be administered diluted in the culture medium or, <i>in vivo</i> via intraperitoneal injection, subcutaneous osmotic pump implants (Tesfaiqzi et al. 2004) or in drinking water (Moser et al. 2004). Clone BU1/75 (ICR1) has been used to detect CldU to study the speed of DNA replication fork (Bugler et al. 2010), in the detection of CldU label retaining stem cells (Kimoto et al. 2008) and label retaining neurons (Murata et al. 2011).
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Flow Cytometry	Use 10ul of the suggested working dilution to label 10 ⁶ cells in 100ul.
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**Recommended
Protocol**

FLOW CYTOMETRY ANALYSIS

Prepare the following solutions before proceeding:

Phosphate buffered saline (PBS)
2N HCl containing 0.5% Triton X-100
PBS containing 0.05% Tween-20
PBS containing 1% BSA (PBS/BSA)
10mg/ml Propidium iodide (PI)
0.1M Na₂B₄O₇, pH 8.5

1. Add BrdU to the cell suspension in culture medium to a final concentration of 10 µmol/L and incubate for 30 minutes in a CO₂ incubator at 37°C.
2. Wash cells twice with PBS/BSA by centrifuging at 500g for 10 minutes, decant supernatant and resuspend in a minimum volume of PBS.
3. Add cells slowly into 5ml of 70% ethanol at -20°C, mixing continuously (vortex preferred). Incubate on ice for 30 minutes.
4. Centrifuge at 500g for 10 minutes, decant supernatant, and resuspend cell pellet.
5. Add 2ml of 2N HCl containing 0.5% Triton X-100 and incubate the cells for 30 minutes at room temperature (preferably on a rocking platform).
6. Centrifuge at 500g for 10 minutes, decant supernatant and resuspend in 3 ml of 0.1M Na₂B₄O₇, pH 8.5.
7. Centrifuge at 500g for 10 minutes, decant supernatant and resuspend the cells in PBS/BSA + 0.05% Tween-20. Adjust cell concentration to 1 x 10⁷/ml.
8. Aliquot 100ul of cell suspension into required number of 12 x 75mm tubes.
9. Incubate the cells with the BrdU antibody at the recommended dilution for 30 minutes at room temperature.
10. Add 2 ml of PBS/BSA and centrifuge the cells at 1000rpm for 5 minutes.
11. If a secondary antibody layer is required then decant the supernatant and incubate the cells with the secondary antibody for 30 minutes at room temperature. If no secondary antibody layer is required then proceed to step 13.
12. Wash the cells after the secondary antibody layer by repeating step 10.
13. Decant the supernatant and add 1ml of PBS containing 10µg/ml PI (Dilute the 10mg/ml solution of PI 1/1000 in a suitable volume of PBS).
14. Analyze cells by flow cytometry following the manufacturer's instructions. The PI should be read on the appropriate channel set to the Peak/Area and not log scale.

For Flow Cytometry references, please visit the following website:
www.bio-rad-antibodies.com/support/brdu_antibody_clone_bu1_75_icr1_references-985.html

IMMUNOHISTOLOGY

Formalin-fixed paraffin-embedded tissue sections:

Clone BU1/75 (ICR1) can be used for labeling paraffin-embedded tissue sections fixed in formalin. Denaturation of the DNA is critical for successful staining of BrdU. This can be achieved by exposing cells to heat, or acid. For heat-induced epitope retrieval, 10mM citrate buffer pH6.0 is recommended. Alternatively, a 30 min incubation in 2M HCl can be performed. The HCl must then be neutralized for 2 min with 0.1 M Na₂B₄O₇. Pretreatment of tissues with proteinase K should be avoided.

For Immunohistology references, please visit the following website:
www.bio-rad-antibodies.com/support/brdu_antibody_clone_bu1_75_icr1_references-985.html

Storage

Store at +4°C or at -20°C if preferred.

This product should be stored undiluted.

Storage in frost free freezers is not recommended. Avoid repeated freezing and thawing as this may denature the protein. Should this product contain a precipitate we recommend microcentrifugation before use.

Shelf Life

18 months from date of despatch.

Health And Safety Information

Material Safety Datasheet Documentation #10040 available at:
<https://www.bio-rad-antibodies.com/uploads/MSDS/10040.pdf>

Regulatory

For research purposes only

Related Products

Recommended Secondary Antibodies

Rabbit Anti Rat IgG (STAR16...)	DyLight®800
Goat Anti Rat IgG (STAR73...)	RPE
Rabbit Anti Rat IgG (STAR21...)	HRP
Rabbit Anti Rat IgG (STAR17...)	FITC
Goat Anti Rat IgG (MOUSE ADSORBED) (STAR71...)	DyLight®549 , DyLight®649 , DyLight®800
Rabbit Anti Rat IgG (STAR20...)	RPE
Goat Anti Rat IgG (STAR131...)	Alk. Phos. , Biotin
Goat Anti Rat IgG (STAR72...)	HRP
Goat Anti Rat IgG (STAR69...)	FITC

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'M273471:150819'

Printed on 08 Jun 2017

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