Datasheet: OBT0030G

Description:	RAT ANTI BrdU
Specificity:	BrdU
Other names:	5-BROMODEOXYURIDINE
Format:	Purified
Product Type:	Monoclonal Antibody
Clone:	BU1/75 (ICR1)
Isotype:	IgG2a
Quantity:	0.25 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
Buffer Solution	Phosphate buffered saline
Preservative Stabilisers	0.09% Sodium Azide 25% Glycerol
Approx. Protein Concentrations	IgG concentration 0.5 mg/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 *in vivo* and *in vitro*. 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, *in vivo* via intraperitoneal injection, subcutaneous osmotic pump implants (Tesfaigzi *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).

Flow Cytometry

Use 20ul of the suggested working dilution to label 10^6 cells in 100ul.

References

1. Vanderlaan, M. & Thomas, C.B. (1985) Characterization of monoclonal antibodies to bromodeoxyuridine. Cytometry. 6: 501-505.
2. Ghiringelli, F. *et al.* (2005) Tumor cells convert immature myeloid dendritic cells into TGF-beta-secreting cells inducing CD4+CD25+ regulatory T cell proliferation. J. Exp. Med. 202: 919-929.
3. Dolbeare, F. (1995) Bromodeoxyuridine: a diagnostic tool in biology and medicine, Part I: Historical perspectives, histochemical methods and cell kinetics. Histochem. J. 27: 339-369.
4. Das, G. *et al.* (2009) Cyclin D1 fine-tunes the neurogenic output of embryonic retinal progenitor cells. Neural Dev. 4: 15.
5. Nakhai, H. *et al.* (2008) Conditional ablation of Notch signaling in pancreatic development. Development. 135: 2757-65.
6. Ghai, K. *et al.* (2010) Notch signaling influences neuroprotective and proliferative properties of mature Müller glia. J Neurosci. 30: 3101-12.
7. Amador-Arjona, A. *et al.* (2011) Primary cilia regulate proliferation of amplifying progenitors in adult hippocampus: implications for learning and memory. J Neurosci. 31: 9933-44.
8. Bugler, B. *et al.* (2010) Unscheduled expression of CDC25B in S-phase leads to replicative stress and DNA damage. Mol Cancer. 9: 29.
9. Gonzalo-Gobernado, R. *et al* (2009) Mobilization of neural stem cells and generation of new neurons in 6-OHDA-lesioned rats by intracerebroventricular infusion of liver growth factor. J Histochem Cytochem. 57: 491-502
10. Xu, Q. *et al.* (2010) Sonic hedgehog signaling confers ventral telencephalic progenitors with distinct cortical interneuron fates. Neuron. 65: 328-40.
11. Zhang, J. *et al.* (2010) A powerful transgenic tool for fate mapping and functional analysis of newly generated neurons. BMC Neurosci. 11: 158.
12. Bonzo, J.A. *et al.* (2012) Suppression of hepatocyte proliferation by hepatocyte nuclear factor 4α in adult mice. J Biol Chem. 287 (10): 7345-56.
13. Knopf, F. *et al.* (2011) Bone regenerates via dedifferentiation of osteoblasts in the zebrafish fin. Dev Cell. 20: 713-24.
14. Grotek, B. *et al.* (2013) Notch signaling coordinates cellular proliferation with differentiation during zebrafish fin regeneration. Development. 140: 1412-23.
15. Kim, T.H. & Shivdasani, R.A. (2011) Genetic evidence that intestinal Notch functions vary regionally and operate through a common mechanism of Math1 repression. J Biol Chem. 286

(13): 11427-33.
16. Kroehne, V. *et al.* (2011) Regeneration of the adult zebrafish brain from neurogenic radial glia-type progenitors. Development. 138: 4831-41.
17. Liu, M.T. *et al.* (2009) 5-HT4 receptor-mediated neuroprotection and neurogenesis in the enteric nervous system of adult mice. J Neurosci. 29: 9683-99.
18. Lundgren, O. *et al.* (2011) Intestinal epithelial stem/progenitor cells are controlled by mucosal afferent nerves. PLoS One. 6: e16295.
19. Muja, N. *et al.* (2011) Neural precursors exhibit distinctly different patterns of cell migration upon transplantation during either the acute or chronic phase of EAE: a serial MR imaging study. Magn Reson Med. 65 (6): 1738-49.
20. Puverel, S. *et al.* (2011) RanBPM is essential for mouse spermatogenesis and oogenesis. Development. 138: 2511-21.
21. Sabo, J.K. *et al.* (2011) Remyelination is altered by bone morphogenic protein signaling in demyelinated lesions. J Neurosci. 31: 4504-10.
22. Scheys, J.O. *et al.* (2011) Evidence of adrenal failure in aging dax1-deficient mice. Endocrinology. 152: 3430-9.
23. Wang, W. *et al.* (2013) Extracellular signal-regulated kinase 5 (ERK5) mediates prolactin-stimulated adult neurogenesis in the subventricular zone and olfactory bulb. J Biol Chem. 288: 2623-31
24. Zemans, R.L. *et al.* (2013) Role of β-catenin-regulated CCN matricellular proteins in epithelial repair after inflammatory lung injury. Am J Physiol Lung Cell Mol Physiol. 304 (6): L415-27.
25. Zimmerman KM *et al.* (2013) Diminished origin-licensing capacity specifically sensitizes tumor cells to replication stress. Mol Cancer Res. 11 (4): 370-80.
26. Maden, M. *et al.* (2013) Proliferation zones in the axolotl brain and regeneration of the telencephalon. Neural Dev. 8: 1.
27. Morita-Takemura Shoko *et al.* (2016) Changes in endothelial cell proliferation and vascular permeability after systemic lipopolysaccharide administration in the subfornical organ Journal of Neuroimmunology. Jul 1 [Epub ahead of print]
28. Sellner, S. *et al.* (2016) Microglial CX3CR1 promotes adult neurogenesis by inhibiting Sirt 1/p65 signaling independent of CX3CL1. Acta Neuropathol Commun. 4 (1): 102.
29. Morrone, C.D. *et al.* (2016) Effects of Neurotrophic Support and Amyloid-Targeted Combined Therapy on Adult Hippocampal Neurogenesis in a Transgenic Model of Alzheimer's Disease. PLoS One. 11 (10): e0165393.
30. Pepe, G. *et al.* (2017) Self-renewal and phenotypic conversion are the main physiological responses of macrophages to the endogenous estrogen surge. Sci Rep. 7: 44270.
31. Howe, J.R. 6th *et al.* (2017) MiR-338-3p regulates neuronal maturation and suppresses glioblastoma proliferation. PLoS One. 12 (5): e0177661.

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 $Na_2B_4O_7$, 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 CO_2 incubator at 37°C.

2. Wash cells twice with PBS/BSA by centrifuging at 500g for 10 minutes, decant supernatant

and resuspend in a miniumum 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 $Na_2B_4O_7$, 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 10^7/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 45 minutes at room temperature, or overnight at 4°C.

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 by repeating step 10.

13. Decant off 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. Analyse 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 $Na_2B_4O_7$. 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 #10049 available at: https://www.bio-rad-antibodies.com/uploads/MSDS/10049.pdf
Regulatory	For research purposes only

Related Products

Recommended Secondary Antibodies

Rabbit Anti Rat IgG (STAR21...) HRP
Rabbit Anti Rat IgG (STAR17...) FITC
Rabbit Anti Rat IgG (STAR20...) RPE

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