iFluor™ 488 Conjugated Anti-BrdU Antibody [PSH0-18]

HA720186F

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Species independent
Applications:	IF-Cell, IF-Tissue, FC
Clone number:	PSH0-18

Description: Bromodeoxyuridine (5-bromo-2'-deoxyuridine, BrdU, BUdR, BrdUrd, broxuridine) is a synthetic nucleoside analogue with a chemical structure similar to thymidine. BrdU is commonly used to study cell proliferation in living tissues and has been studied as a radiosensitizer and diagnostic tool in people with cancer. During S phase of the cell cycle (when DNA replication occurs), BrdU can be incorporated in place of thymidine in newly synthesized DNA molecules of dividing cells. Cells that have recently performed DNA replication or DNA repair can be detected with antibodies specific for BrdU using techniques such as immunohistochemistry or immunofluorescence. BrdU-labelled cells in humans can be detected up to two years after BrdU infusion. Because BrdU can replace thymidine during DNA replication, it can cause mutations, and its use is therefore potentially a health hazard. However, because it is neither radioactive nor myelotoxic at labeling concentrations, it is widely preferred for in vivo studies of cancer cell proliferation. However, at radiosensitizing concentrations, BrdU becomes myelosuppressive, thus limiting its use for radiosensitizing. BrdU differs from thymidine in that BrdU substitutes a bromine atom for thymidine's CH3 group. The Br substitution can be used in X-ray diffraction experiments in crystals containing either DNA or RNA. The Br atom acts as an anomalous scatterer and its larger size will affect the crystal's X-ray diffraction enough to detect isomorphous differences as well. Bromodeoxyuridine releases gene silencing caused by DNA methylation. DNA with BrdU transcribes as usual DNA, with guanine included into RNA as a complement to BrdU.

Conjugate:	iFluor™ 488, Ex: 491nm; Em: 516nm.
Immunogen:	BrdU-OVA
Positive control:	BrdU treated NIH/3T3, BrdU treated mouse embryo tissue, BrdU treated HeLa.
Subcellular location:	Nucleus.
Recommended Dilutions:	
IF-Cell	1:50
IF-Tissue	1:200
FC	1:100
Storage Buffer:	Preservative: 0.02% Sodium azide Constituents: 30% Glycerol, 1% BSA, 68.98% PBS.
Storage Instruction:	Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.
Purity:	Protein A affinity purified.

Images

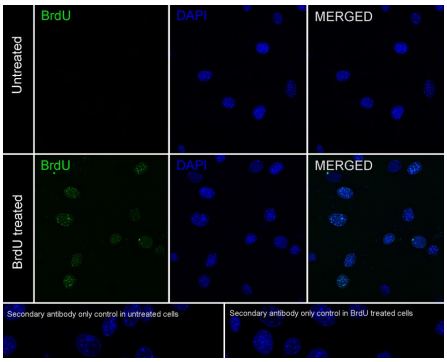

Fig1: Immunocytochemistry analysis of NIH/3T3 cells (Untreated / Brdu treated) labeling BrdU with Rabbit anti-BrdU antibody (HA720186F) at 1/50 dilution.

Cells were fixed in 70% ethyl alcohol for 5 minutes at room temperature, then subjected to acid hydrolysis using 2M HCL in TBST for 30 minutes at room temperature. permeabilized with 0.1% Triton X-100 in PBS for 15 minutes, and then blocked with 2% BSA for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-BrdU antibody (HA720186F, iFluor™ 488) at 1/50 dilution in 2% negative goat serum overnight at 4 ℃. Nuclear DNA was labelled in blue with DAPI.

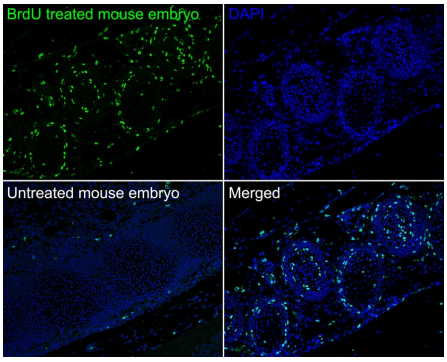

Fig2: Immunofluorescence analysis of paraffin-embedded BrdU treated/untreated mouse embryo tissue labeling BrdU with Rabbit anti-BrdU antibody (HA720186F) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA720186F, green) at 1/200 dilution overnight at 4 ℃, washed with PBS. Nuclei were counterstained with DAPI (blue).

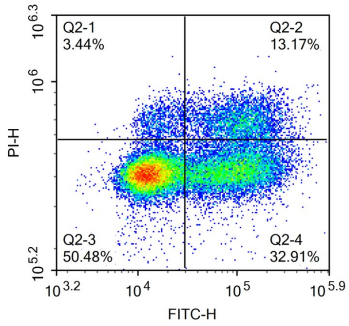

Fig3: Dot plot showing BrdU treated HeLa cells stained with HA720186F. Cells were incubated with 10 µM BrdU for 24 hours prior to being harvested, washed twice in 1x PBS and fixed in 70% ethanol at 4℃ for 30 minutes. Once fixed, pellets were acid denatured with 2M HCl for 30 minutes at room temperature and then neutralised with borate buffer (0.1M, pH8.5) for 15 minutes.

Samples were washed and incubated in 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (HA720186F, 10µg/ml) for 30 min at room temperature.

PI was added to cells 15 min prior to data acquisition.

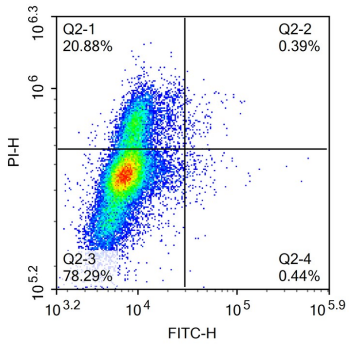

Fig4: Dot plot showing untreated HeLa cells (Negative) stained with HA720186F. Cells were incubated without 10 µM BrdU for 30 minutes prior to being harvested, washed twice in 1x PBS and fixed in 70% ethanol at 4℃ for 30 minutes. Once fixed, pellets were acid denatured with 2M HCl for 30 minutes at room temperature and then neutralised with borate buffer (0.1M, pH8.5) for 15 minutes.

Samples were washed and incubated in 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (HA720186F, 10µg/ml) for 30 min at room temperature.

Background References

1. Wang T et al. Unexpected BrdU inhibition on astrocyte-to-neuron conversion. Neural Regen Res. 2022 Jul
2. Yu J et al. BrdU Incorporation Assay to Analyze the Entry into S Phase. Methods Mol Biol. 2022