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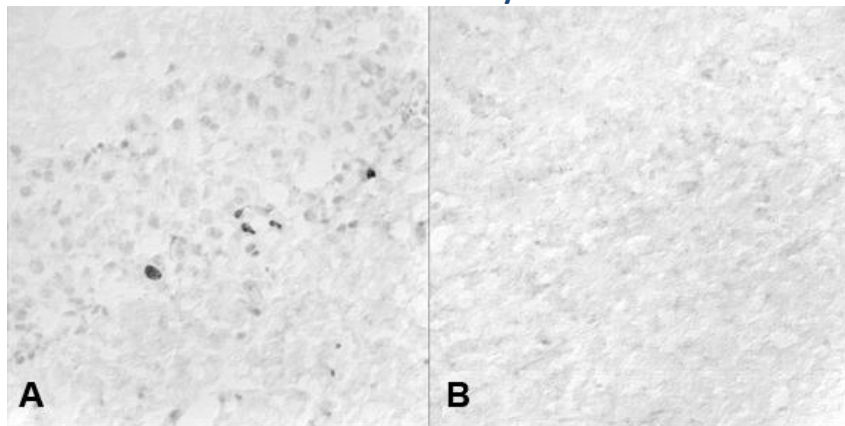
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BrdU Antibody-W:

General:

This antibody reacts against the synthetic thymidine analog BrdU, which when injected into an animal is incorporated during the S phase of the cell cycle into newly synthesized DNA strands of actively proliferating cells. The number of cells that survive can be measured by immunocytochemistry. As an outstanding tool to assess the proliferation state of a group of cells, it has become vital to drug discovery research, especially with cancer therapeutics and ascertaining the health of cells during ADME/Tox studies. BrdU Antibody-W is a polyclonal raised in rabbit against BrdU conjugated to bovine serum albumin (BSA). Our antibody has been used for immunocytochemistry in rat brain tissue. It will be sold with filter paper absorbed with BrdU for blocking. This allows the researcher to confirm the specific signal.

Characterization of the BrdU Antibody-W



A. BrdU positive cells in dentate gyrus (60X) of an adult rat previously injected with BrdU.
Photo taken on confocal microscope

B. BrdU block in dentate gyrus

Specificity was ascertained by performing a blocking study. Adjacent sections from the hippocampus of an adult rat that had been previously injected with BrdU were incubated with the BrdU Antibody-W alone (A-60X) or with BrdU Antibody-W preincubated with BrdU (B-60X). Note labeling in A and the absence of labeling in B, indicating specificity of the antibody blocking.

Storage and Preparation:

The vial you will receive contains approximately 200µl at a dilution of 1/100. BrdU Antibody-W can be further diluted to at least 1/30,000 in BSA diluent (50mM KPBS, 0.4% Triton, 1% BSA, 1% NGS).

The antibody should be stored at -20C. You will receive the antibody unfrozen. Refreeze it unless you intend to use it within 48 hours. Avoid refreezing the antibody multiple times.

Also included is filter paper absorbed with BrdU for blocking. 500µl of the diluted antibody can be added to the tube to achieve a 10 µM block. Incubate for at least 1hour at room temperature (RT) before applying to tissue. The antibody blocks specifically with BrdU and does not block with other peptides absorbed on filter paper.

Protocol:

Fresh frozen 10-30 micron sections are fixed in 4% paraformaldehyde (FA) for 1 hour at RT, washed in phosphate buffered saline (PBS), then treated with 50% Formamide/2X SSC (300 mM sodium chloride and 30 mM sodium citrate) at 65C for 2 hours. The sections are washed in 2X SSC, incubated in 2N HCL at 37C for 30 minutes, and then placed directly in 100mM Sodium Borate (pH 8.5) for 10 minutes at RT. The sections are washed in PBS and incubated with 0.1 to 0.3% H₂O₂ in PBS for 10 to 30 minutes. The tissue is again washed in PBS, then treated with an Avidin/Biotin blocking kit (Vector-cat #SP-2001), washed and incubated with BSA diluent for 1 hr at RT. The BrdU Antibody-W (1/30,000) is applied to the tissue overnight at RT. After washing in PBS the sections are incubated in Biotinylated GAR at 1/1000 (Vector labs-cat#BA1000) for 1hr at RT, washed and then incubated in Avidin/Biotin at 1/1000 (Vector labs-cat#PK6100) for 1hr at RT. After washing the signal is visualized with Diaminobenzidine (0.04%) in 0.1M Sodium Acetate with 0.06% H₂O₂ for six minutes (Nickel Chloride at 2.5% may also be added). Sections are then water washed (counterstained if desired), dehydrated and cover slipped.

Publications:

Garcia-Fuster et al, in preparation: Individual differences in adult hippocampal neurogenesis following experimenter administration of cocaine.