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Protocol for BrdU Labeling of Proliferating Cells

When added to culture medium, BrdU is incorporated into the DNA of cells that are in the S-phase of the cell cycle. A relatively short exposure time (2-4 hr.) will allow very few BrdU-containing cells to progress through M-phase. This will minimize the number of labeled 'double nuclei' and will permit a more accurate quantification of cell proliferation. Cellular immunoreactivity for BrdU should be *strictly* nuclear; any cytoplasmic labeling is suspect and may indicate a problem with the tissue and/or protocol.

(1) Add BrdU to culture medium, for a final concentration of 3 µg/ml. Incubate tissue at 37^{o} C for 2-4 hrs.

(2) Fix for 30-60 min. with 4% paraformaldehyde (in 0.1M PB).

(3) Rinse 5x with PBS, over ~20 min.

(4) Treat fixed cultures for 30 min. in 2N HCl. If preparing 2N HCl from a 12N stock solution, simply dilute 1:5 in dH_2O. This treatment separates DNA into single strands (i.e., 'denatures' DNA), so that the primary antibody has access to the incorporated BrdU. Make sure that the cultures are *completely* submerged in the HCl (they sometimes tend to float).

(5) Rinse 3x with PBS, over ~10 min.

(6) Block nonspecific epitopes by incubating tissue for 1-2 hr. in 5% normal horse serum (NHS, in PBS with 0.2% Triton X-100)

(7) Incubate in anti-BrdU primary antibody. The dilution of the antibody will depend on the supplier's recommendations. Anti-BrdU antibodies are generally mouse IgG's, although some goat polyclonals are also available. The primary antibody solution should be made in PBS and contain 2% NHS and 1% Triton X-100. Incubate tissue overnight.

(8) The next day, rinse 5x with PBS, over ~20 min.

(9) Incubate in secondary antibody (usually anti-mouse IgG, diluted in PBS with 0.2% Triton X-100) for 2 hr. at room temperature. It is also helpful to add DAPI to this solution, in order to label *all* cell nuclei.

(10) Mount tissue on microscope slide, with the apical surface facing upwards.