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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 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 !g/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 submerged in the HCl (they sometimes tend to float).

(5)