Evaluation of Click Chemistry Based Alternative to BrdU Antibody Labeling in Tissue and Cultured Cells Using Fluorescence Microscopy and Flow Cytometry

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Introduction
Two methods for detection of cell proliferation based on BrdU or EdU (5-ethyl-2’-deoxyuridine) nucleoside incorporation were evaluated in animal models and cell lines for sensitivity, time requirement and ease of use. Two types of animal models were used in this evaluation; estrogen induced proliferation in the rat model followed by a 2 hour pulse of nucleoside analog, and labeling of stem cell derived cells with a 3 or 15 day nucleoside pulse in the songbird adult neurogenesis model. In cultured cells, using A549, HeLa, and Jurkat cells, compatibility with antibody labeling was included in the parameters of comparison.

Results and Conclusions

Figure 2A – Characterization of DNA content, cell proliferation (EdU or BrdU labeling), endogenous cyclin B1 intensity using automated imaging cytometry. HeLa cells in 96 well plates were treated for 24 hours with the 594 nm cell cycle blocker nocodazole (170 µM) then labeled with 10 µM EdU for 5 min or 15 min before processing for detection. Click labeling of EdU (row 1) was compared to 2 protocols for BrdU detection from commercially available reagent kits; one involving inversion antibody labeling (row 2), and another involving a DNA(ds) treatment (row 3). Anti-click-1 B1 and Hoechst 33342 were included in the labeling procedures. Fluorescence micrographs show 3 color panels collected during automated image acquisition.

Figure 2B – Automated image acquisition and analysis performed with an Aramaycn VTII (Thermo Fisher / Cellomics, Pittsburgh, PA) were followed by data examination and VTI (Thermo Fisher / Cellomics, Pittsburgh, PA) were followed by data examination and plotted using Parno (The Chi-Square Works, Inc. Seabeck, WA). The distributions of DNA content (Hoechst 33342 stain), EdU or BrdU nuclear intensity and cyclin B1 nuclear intensity are shown as histogram trellis plots. A split in DNA content profile and decrease in cell proliferation EdU or BrdU incorporation can be revealed in nocodazole treated cells. Anti-cyclin B1 only achieved good signal-background separation in cells processed for click EdU detection (red arrow) but not in cells processed for BrdU detection.

Figure 3 – Labeling of avian model of stem cell derived neurons

Figure 4 – Protocols used for processing rat tissue sections. Paraffin embedded tissue was deparaffinized with standard xylene based protocol. Overnight labeling with BrdU antibody at 4°C was required to obtain a sufficiently bright signal. 2 hour labeling at room temperature with primary anti-BrdU antibody did not give a detectable signal.

Figure 5 – Detection of proliferating rat mammary epithelial cells with estradiol treatment using EdU and BrdU

Figure 6 – rat control group—no estradiol treatment

Figure 7 – Nonspecific signal of Click-IT™ EdU and BrdU

Results and Conclusions

Hormone induced proliferation in rat mammary epithelial tissue labeled with Click-IT™ EdU gave improved sensitivity over BrdU and was simpler, faster and easier to use. Detection of DNA for good BrdU labeling resulted in loss of nuclear staining quality. Overnight labeling with anti-BrdU was required for adequate labeling in tissue. Two hour labeling with anti-BrdU was not sufficient to obtain a strong positive signal. Asian brain model of adult born neurons could be imaged using a shortened protocol of 25 minutes and resulted in a clear signal while the most rapid BrdU protocol required overnight treatment or 2 hours if high background was acceptable. In cultured cells, 5 minute pulses of BrdU or EdU produced detectable signals in adherent cells; however, denaturation requirements for the BrdU protocol drastically reduced its compatibility in multiplex labeling with other antibody probes. Multiplexed detection of cyclin B1 in cultured cells only achieved good signal to noise with EdU protocol.

Figure 8 – Sensitivity of detection using long wavelength Click-IT™ detection reagent

References
2. Rostovtsev, V.V.; Green, L.G.; Fokin, V.V.; Sharpless, K.B. Angew. Chem. Int. Ed. 2002, 41, 2596.