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Immunohistochemistry Protocol for BrdU Antibody (NB500-235)

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Immunohistochemistry protocol for Bromodeoxyuridine (BrdU) Polyclonal Antibody

Original antigenic studies performed by Dr David Stollar, Tufts University, Boston, MA

ABSTRACT

New methods for double and triple colour labeling using monoclonal antibodies to the proliferation-associated markers 5-methly-cytosine, BrdU and Ki67 are described. In order to make incorporated 5-methyl-cytosine or BrdU accessible to most antibodies, mild denaturation of the DNA is needed, and this is usually obtained by exposing the cells to acid or base. This procedure destroys most cellular antigen, including nuclear TdT and Ki67. In this study, we show that fixation in cold methanol instead of 70% ethanol for 30 minutes followed by immersion in 7 x 10-3 N NaOH for 10-15 seconds allows BrdU staining with the simultaneous detection of nuclear cytoplasmic and membrane assigns as well as preservation of morphological detail. This method is optimal for detection of nuclear Ki67 and TdT. These reagents, together with antibodies to membrane assigns can be included in triple colour labeling using second layers conjugated to FITC, TRITC and colloidal gold. With these methods it is now possible to characterize the phenotype of dividing cell populations such as precursors in central lymphoid tissues and germinal centre blasts in peripheral lymphoid organs.

Reference for tissue staining with anti-BrdU
Campana D, Coustan-Smith E., Janossy G., Department of Immunology, Royal Free Hospital School of Medicine, London, UK. J Immunol Methods 107(1):79-88 1988 Feb 24 Double and triple staining methods for studying the proliferative activity of human B and T lymphoid cells Abstract

For best results on unconjugated antibody, use the product at a concentration of 25 to 100 ug/mL. Nearly complete immunoprecipitation was obtained at concentrations of 100 to 500ug/mL. The product has also been tested for utility for staining of bromodeoxyuridine incorporated into DNA of replicating cells. When utilized at a purified concentration of 10 ug/mL, the product is comparable to commercially available monoclonal antibodies commonly used for the same purpose.

A key issue is that it is necessary to denature the DNA so as to expose the base to the antibodies. One protocol is: Sachiko Matsuuraa and Kazuo Suzukia Immunohistochemical Analysis of DNA Synthesis During Chronic Stimulation with Isoproterenol in Mouse Submandibular Gland . Journal of Histochemistry and Cytochemistry, Vol. 45, 1137-1146, Immunohistochemistry DNA-synthesizing cells were detected by peroxidase-anti-peroxidase (PAP) immunostaining with anti-BrdU antibodies by the method of Harms et al. 1986 , which was slightly modified as follows: Sections were treated with 2 N HCl at 60C for 15 min and washed in running water for 15 min. After dehydration in an ethanol series, the sections were etched with xylene (15 min, twice). Nuclear proteins, which mask incorporated BrdU, were digested with 0.025% protease (Type V; Sigma) in PBS at 37C for 10 min, and the sections were then washed with cold PBS three times for 5 min.