Anti-BrdU, formalin grade

Cat. No. 11 170 376 001 50 µg

Anti-BrdU-Fluorescein

Cat. No. 11 202 693 001 50 µg

Anti-BrdU-Peroxidase, Fab fragment

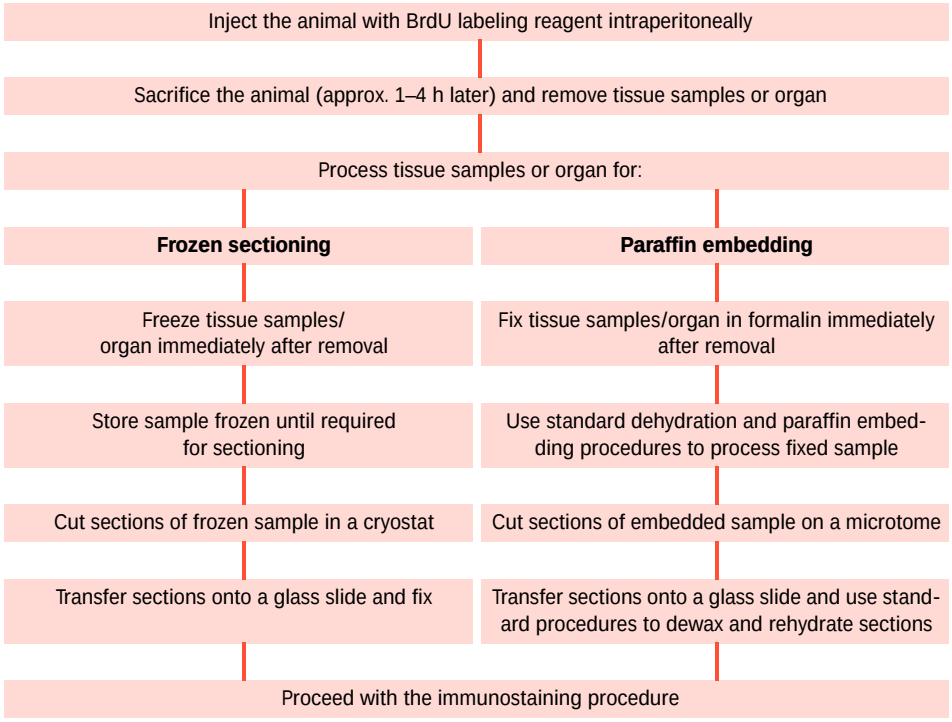
Cat. No. 11 585 860 001 15 units

Type	Monoclonal antibodies, from mouse
Useful for	Detection of BrdU-labeled DNA in proliferating individual cells
Samples	Cultured or freshly isolated cells, tissue explants or sections
Method	Incubation of samples with BrdU, followed by denaturation of DNA, detection of BrdU label with anti-BrdU antibody, and (if necessary) visualization of anti-BrdU antibody with secondary antibody
Time	Variable (depending on sample and antibody used)

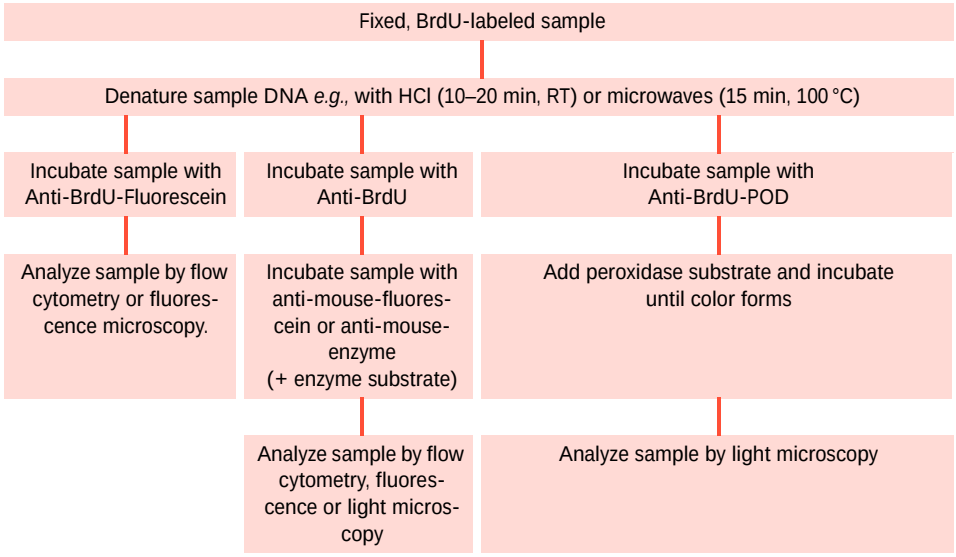
Significance of antibodies: Bromodeoxyuridine (BrdU) is only incorporated into the DNA of proliferating cells. Short periods (15–60 min) of incubation *in vitro* with BrdU will tag only cells going through the S phase of the cell cycle. Alternatively, BrdU can be injected into an animal to label growing cells *in vivo*. Conjugated or unconjugated anti-BrdU antibody may be used to detect proliferating cells or tissues which have been tagged by *in vitro* or *in vivo* BrdU labeling. Depending on the sample and the antibody used, analysis can be by flow cytometry, fluorescence microscopy, or light microscopy.

Test principle: The anti-BrdU antibodies may be used to detect BrdU-labeled DNA in proliferating cells. The procedure involves (Flow Chart 19):

1	**A:** Incubating growing animal tissue or cells *in vitro* with BrdU — or — **B:** Injecting BrdU into whole animals for *in vivo* labeling, then sacrificing the animal and preparing tissue sections. ***Note:*** *Only proliferating cells (cells in S-phase) incorporate BrdU into their DNA.*
2	Fixing BrdU-labeled tissue or cells.
3	Denaturing cellular DNA.
4	Detecting incorporated BrdU with conjugated or unconjugated anti-BrdU monoclonal antibody.
5	(Option) **A:** Localizing unconjugated anti-BrdU antibody with a secondary antibody detection system — or — (Option) **B:** Localizing enzyme-conjugated anti-BrdU antibody with an enzyme substrate.
6	Analyzing the antibody-labeled samples with a flow cytometer, a fluorescence microscope, or a light microscope.



▲ **Flow Chart 18: Assay procedure, *in vivo* labeling of proliferating cells with BrdU.**



▲ **Flow Chart 19: Immunostaining procedure, Anti-BrdU antibody and conjugates.**

Specificity: Conjugated or unconjugated anti-BrdU antibody will bind to BrdU-labeled DNA after the DNA is denatured and partially degraded (*e.g.*, with DNase, acid or microwaves). The antibody specifically recognizes 5-bromo-2'-deoxyuridine; it shows no cross-reactivity with any endogenous cellular components such as thymidine or uridine.

Can be used to assay:

- Cell lines (in adherent or suspension cell culture)
- Freshly isolated cells, or tissue explants labeled with BrdU *in vitro*
- Frozen or paraffin-embedded tissue sections from animals labeled with BrdU *in vivo*.

Typical results: The anti-BrdU antibody has been used to determine the cell cycle position of apoptotic cells[76].

Briefly, the experimental procedure was as follows: Cultured mouse thymocytes were treated with 0.5 µM ionomycin (2 h or 12 h) to induce apoptosis. After treatment, the cells were harvested, fixed in paraformaldehyde and ethanol (two-step fixation), and analyzed for apoptosis and cell cycle position by flow cytometry. As a measure of apoptotic cells, fragmented DNA content was quantitated with the *In Situ* Cell Death Detection Kit, Fluorescein (TUNEL method, according to the kit package insert). Either of two flow cytometric techniques was used to determine the cell cycle position of the cells: 1) Relative DNA content was determined by treating the cells with 5 µg/ml propidium iodide and 200 µg/ml ribonuclease (30 min, room temperature). 2) Cells going through S-phase were identified by labeling with BrdU (10 µM BrdU, 30 min), detection of BrdU-labeled cells with anti-BrdU monoclonal antibody (30 min, 37°C), and visualization of those cells with R-phycoerythrin-conjugated goat anti-mouse antibody (30 min, 37°C). For results, see Figure 78.

Other applications: For examples of how the Anti-BrdU conjugates and the antibodies may be used in the lab, see Appendix, pages 152 and 153.

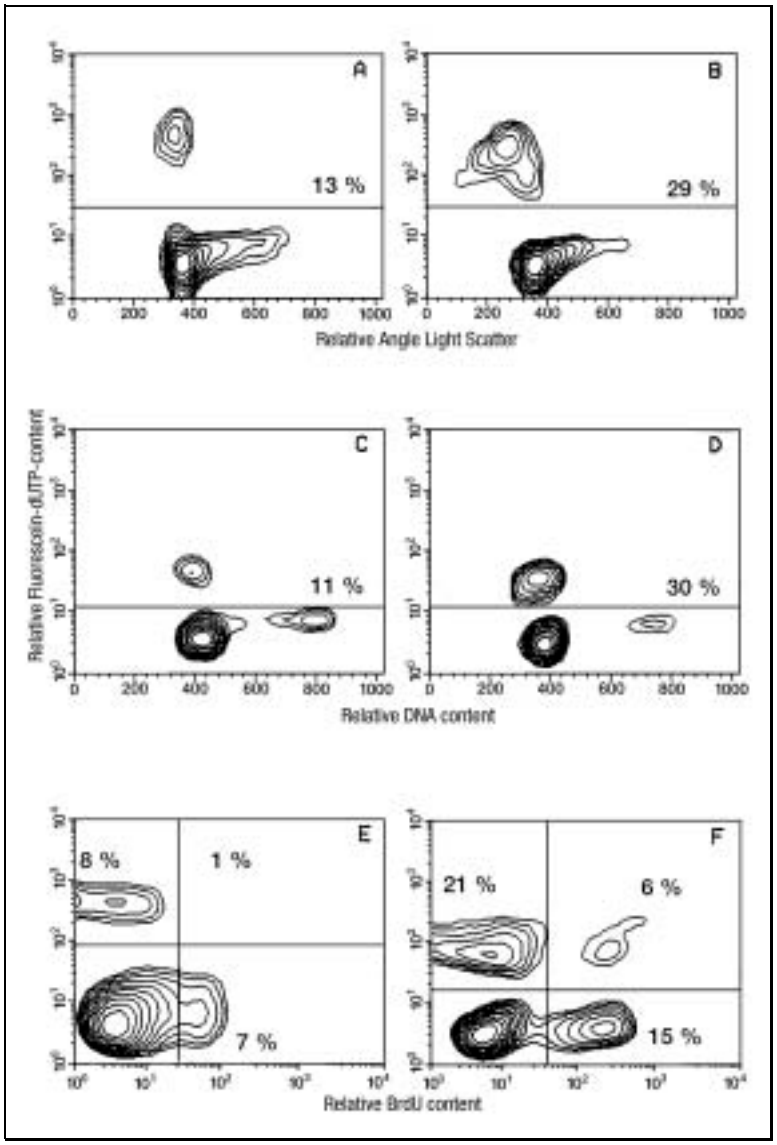


Figure 78: Concomitant flow cytometric analysis of apoptosis and cell cycle position with the anti-BrdU antibody, propidium iodide, and the *In Situ* Cell Death Detection Kit, Fluorescein. Cultured mouse thymocytes were treated with ionomycin (2 h or 12 h) to induce apoptosis. After treatment, the cells were harvested, fixed, and analyzed for apoptosis and cell cycle position by flow cytometry. Histograms A, C, and E show data obtained from cells after 2 h treatment with ionomycin. Histograms B, D, and F show data obtained from cells after 12 h treatment with ionomycin. Histograms A and B show fluorescein intensity (green fluorescence) alone, a measure of DNA fragmentation. Histograms C and D show a two-parameter analysis of fluorescein intensity (green fluorescence, DNA fragmentation) and propidium iodide intensity (red fluorescence, DNA content). Histograms E and F show a two-parameter analysis of fluorescein intensity (green fluorescence, DNA fragmentation) and phycoerythrin intensity (orange fluorescence, BrdU content). The percentage of positive cells is indicated in each panel. [Data from Hanon, E., Vanderplasschen, A. and Pastoret, P.-P. (1996) *Biochemica* **No. 2**, 25–27.]

Result: The ionomycin-treated cells contained about 13% apoptotic cells (histogram A) after 2 h and about 29% apoptotic cells (histogram B) after 12 h exposure. Concomitant analysis of apoptosis and total DNA content (histograms C and D) showed that apoptotic cells contained about as much DNA as cells in G_0/G_1 or early S-phase. Concomitant analysis of apoptosis and BrdU content after 12 h ionomycin treatment (histogram F) showed that 6% of the apoptotic cells went through S phase (that is, were positive for BrdU) while 21% of apoptotic cells remained in G_0/G_1 (that is, were negative for BrdU).

B