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Flow Cytometric Measurement of Total DNA and Incorporated 5-Bromo-2'-deoxy-uridine Using an Enzymatic DNA Denaturation Procedure

Introduction

The flow cytometric detection of replicating cells using monoclonal antibodies directed against thymidine analogs such as 5-bromo-2'-deoxy-uridine (BrdU) has been described (1). Binding of the antibody is only achieved by denaturation of the DNA. This is usually achieved by exposing the cells to acid (2).

In order to develop a flow cytometric measurement technique for total DNA and incorporated BrdU without acidic DNA denaturation, we adapted the protocol of the Boehringer Mannheim 5-Bromo-2'-deoxy-uridine Labeling and Detection Kit I to flow cytometry.

Materials and Methods

Madin Darby Bovine Kidney cells (ATCC CCL 22) were cultured in minimum essential medium (MEM) (GIBCO, Gand, Belgium) containing 5% fetal calf serum (GIBCO, Gand, Belgium). BrdU was added directly to the culture medium to achieve a final concentration of 10 µM. After 1 hr in a CO_2 incubator at 37C° (the incubation period depends on the individual requirements), the cells were harvested and treated for detection of BrdU incorporation.

In order to obtain a cell suspension, the MDBK cells were trypsinized. After centrifugation (10 minutes at 208 g), the cells were resuspended in 200 µl of phosphate-buffered saline (PBS). Three milliliters of 70% ethanol (in 50 mM glycine buffer, pH 2.0) were slowly added drop by drop to the cells while vortexing. After incubation at 4°C for at least 24 hours, the cells were centrifuged and washed with PBS. Cells (10^6) were then resuspended in 300 µl of the anti-BrdU working solution supplied in the BrdU Labeling and Detection Kit I. This solution contains mouse monoclonal antibody anti-BrdU, and nucleases for DNA denaturation.

After incubation at 37°C for 1 hour, the cells were centrifuged (10 minutes, 208 g) and resuspended in 200 µl of the kit's anti-mouse-Ig-fluorescein working solution. After a 30 min incubation at 37°C in the dark, the cells were centrifuged and resuspended in 1 ml of PBS containing 5 µg of propidium iodide (PI). The cells were then analyzed for green (fluorescein isothiocyanate, measuring relative BrdU content) and red (PI, measuring relative DNA content) fluorescences by flow cytometry.

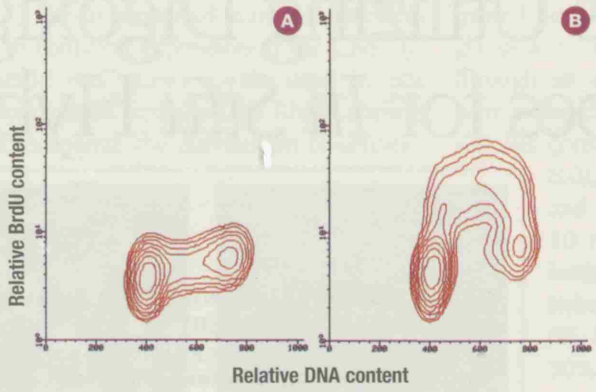

Figure 1 **Measurement of total DNA and BrdU incorporation by flow cytometry.** (A) Control cells were not pulsed with BrdU. (B) Exponentially growing Madin Darby Bovine Kidney cells were pulsed with BrdU for one hour. All cells were then treated as described in the Materials and Methods in order to measure total DNA and BrdU incorporation by flow cytometry.

Results and Discussion

Figure 1 shows the results obtained after cell treatment according to the procedure described in this paper (A cells were not pulsed with BrdU, B cells were pulsed with BrdU). These results were comparable with those obtained following the conventional acidic method (data not shown). Although the two treatments gave similar results in terms of the percentage of cells in the S phase, the counterstaining of cells with propidium iodide showed a higher coefficient of variation for the G_0/G_1 peak when using the DNase method (3.9%) than when using the acid-treatment method (2.7%).

The denaturation of DNA using HCl results in the destruction of cell integrity, including cell morphology and cell markers. Moreover, this acidic treatment excludes the use of fluorochromes of the phycobiliprotein family, such as phycoerythrin and allophycocyanin, before cell fixation (3). Conversely, DNase digestion does not affect cell integrity or the properties of these fluorochromes, which, in association with fluorescein isothiocyanate (FITC), permit dual- or three-color immunofluorescence analysis using single or dual laser beams. ■

Product	Cat. No.	Size
5-Bromo-2'-deoxy-uridine Labeling and Detection Kit I	1296 736	100 assays

Also Available	Cat. No.	Size
Anti-BrdU-POD for direct detection of incorporated BrdU	1585 860	15 U

References

1. Gratzner, H. G. (1982) *Science* **218**:474-475.
2. Dolbeare, F. *et al.* (1983) *Proc. Natl. Acad. Sci. USA* **80**:5573-5577.
3. Carayon, P. and Bord, A. (1992) *J. Immunol. Methods* **147**:225-230.