BD Pharmingen™ Proliferation Kits for cell cycle analysis

Features

Convenient, easy-to-use kits measure cell proliferation in fixed cells.

Two types of kits, which are optimized for either multicolor panel flexibility or quick turnaround on results, fit a variety of experimental needs.

Kits and single vials are available in a myriad of colors, allowing for flexibility in multicolor panel design.

Kits can be used in imaging applications.

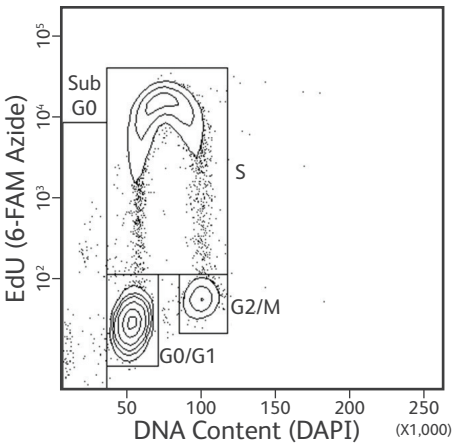


Figure 1. Cell cycle analysis of proliferating HeLa cells using EdU

HeLa cells (ATCC, CCL-2) were pulsed with 10 µM EdU for 1 hour, and then fixed, permeabilized, and stained with 6-FAM azide and BD Pharmingen™ DAPI Solution (Cat. No. 564907) according to the recommended assay procedure for the BD Pharmingen™ 488 EdU Click Proliferation Kit (Cat. No. 565455). Different compartments of the cell cycle can be clearly distinguished by DNA content and EdU incorporation. Plots were derived from gated events based on light scattering characteristics of HeLa cells. Flow cytometry was performed on a BD LSRFortessa™ cell analyzer.

Accurate determination of cell cycle status

Bromodeoxyuridine (BrdU) and ethynyl deoxyuridine (EdU) are synthetic thymidine analogs that are incorporated into newly synthesized DNA by proliferating cells. These thymidine analogs can then be used as measures of de novo DNA synthesis. BrdU is detected by the use of a specific antibody against BrdU, which can be detected by flow cytometry or imaging. EdU, however, is detected by a click chemistry reaction between the ethynyl group of EdU and a fluorescent probe containing an azide moiety. In this reaction, the alkyne and azide groups react selectively to produce a fluorescent, covalently linked product that can also be detected by flow cytometry or imaging.

In both cases, the resulting fluorescence intensity of each cell is proportional to the amount of de novo DNA synthesis that occurred during the incubation period with the thymidine analog. By combining either probe with a DNA content dye, such as 7-AAD or DAPI, two-color flow cytometry can be used to determine the cell cycle phase (G0/G1, S, or G2/M) of the cells (Figures 1 and 2).

BD

Two categories of kits enable flexibility in experimental design

Both BrdU and EdU kits are often used to generate information about cell cycle status (Table 1). When time is constrained, the shorter EdU protocol can provide equivalent information in less time. However, because the EdU protocol requires the use of copper, multiplexing EdU cell cycle analysis with PE-conjugated antibodies requires protocol optimization due to the quenching of PE by copper. In this case, BD Life Sciences provides BrdU flow kits for ease of multiplexing.

Table 1. BrdU and EdU kit information

Protocol	Approximate time*	Multicolor information	Bioimaging/immunofluorescence†
BrdU	3–5 hours	– Antibodies may be co-stained at the same time as BrdU detection. – Compatible with all BD Life Sciences fluorochromes	Yes
EdU	2–3 hours	– Antibodies should be stained in a separate step from EdU detection. – May require optimization for costaining with PE	Yes

*Assay time includes incubation with thymidine analogs.

†See ordering information on page 3 for more information.

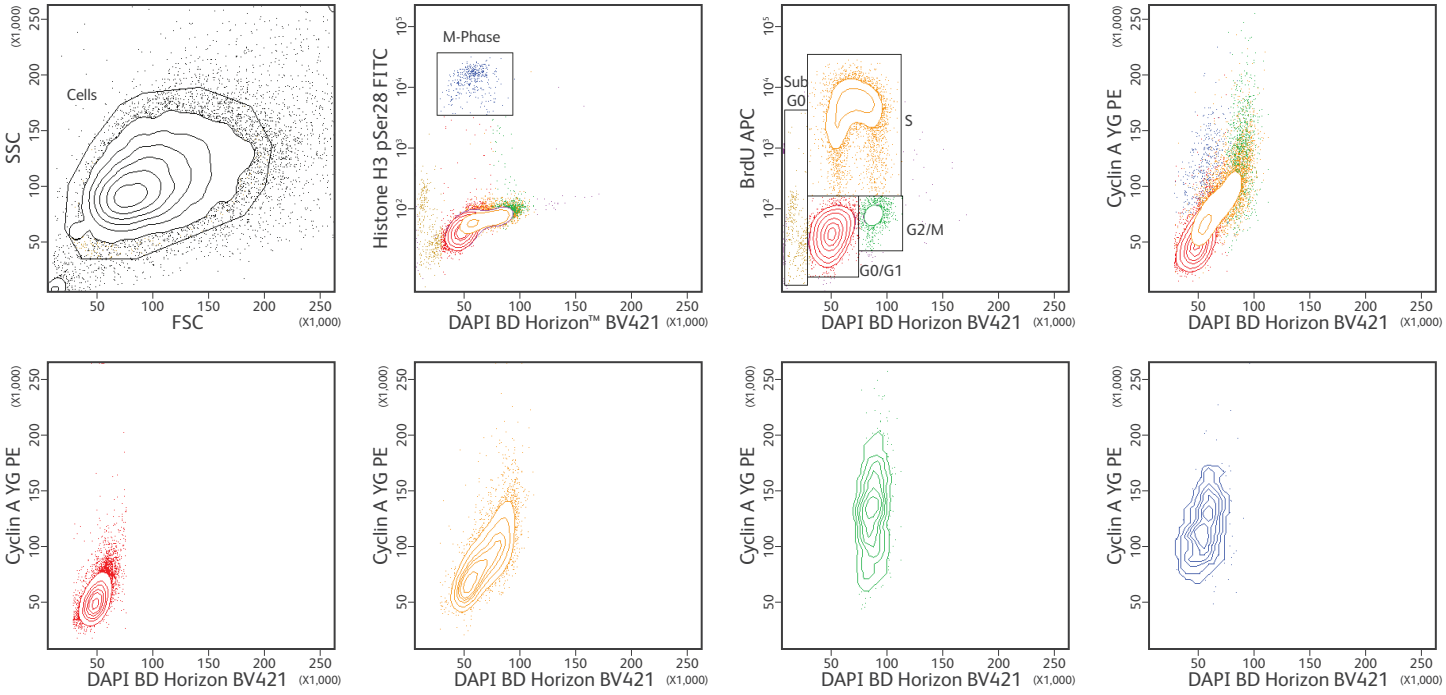


Figure 2. Cell cycle and cyclin analysis in HeLa cells using BrdU

HeLa cells were pulsed with BrdU and then fixed and permeabilized according to the BD Pharmingen™ APC BrdU Flow Kit protocol (Cat. No. 557892). Cells were then stained with APC anti-BrdU, Alexa Fluor® 488 anti-Histone H3 pS28 (Cat. No. 558610), and PE anti-Cyclin A (Cat. No. 550913) according to the kit protocol. After washing, cells were stained with 1 µg/mL BD Pharmingen DAPI Solution for DNA content. Cyclin A expression can be seen to increase over the course of the cell cycle, peaking in the G2 and M phases (overlay, top row; individual compartments, bottom row). Plots were derived from gated events based on light scattering characteristics of HeLa cells. Flow cytometry was performed on a BD LSRFortessa cell analyzer.

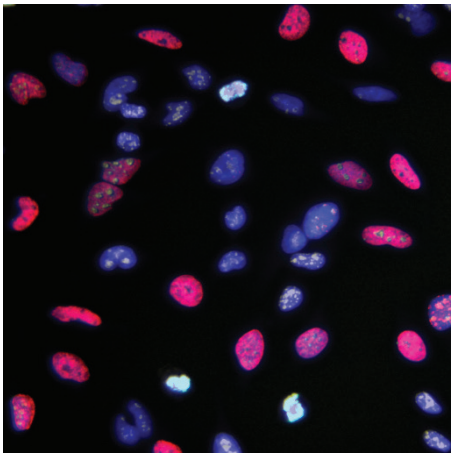


Figure 3. Bioimaging of proliferating HeLa cells using EdU

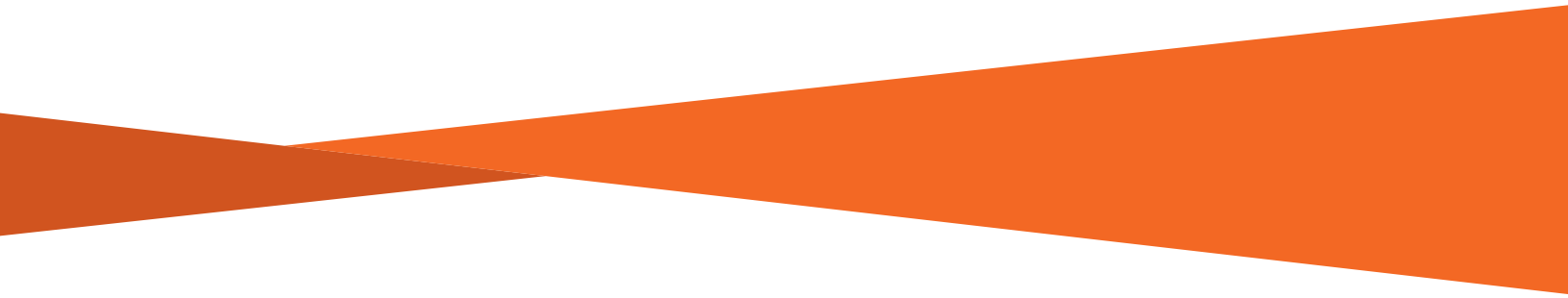
HeLa cells were pulsed with 10 µM EdU for 1 hour, and then fixed, permeabilized, and stained with Eterneon™ Red azide (pseudo-colored red) from the BD Pharmingen™ 647 EdU Click Proliferation Kit (Cat. No. 565456). Cells were washed twice and stained with Alexa Fluor® 488 Rat Anti-Histone H3 (pS28) (pseudo-colored green, Cat. No. 558610) and Alexa Fluor® 555 Mouse Anti-Ki-67 (pseudo-colored yellow, Cat. No. 558617). Nuclei were counterstained with BD Pharmingen DAPI Solution (pseudo-colored blue). Eterneon Red appears magenta when colocalized with DAPI. Histone H3 (pS28) appears white when colocalized with DAPI and Ki-67. S-phase cells can be identified by staining with Eterneon Red and Ki-67, M-phase cells can be identified by staining with Histone H3 (pS28) and Ki-67, and G1/G2-phase cells can be identified by staining with Ki-67 only.

Ordering information

Description*	Application†	Test size	Cat. No.
BD Pharmingen™ 488 EdU Click Proliferation Kit	IC/FCM, Bioimg	50	565455
BD Pharmingen™ 647 EdU Click Proliferation Kit	IC/FCM, Bioimg	50	565456
BD Pharmingen™ APC BrdU Flow Kit	IC/FCM	50	552598
BD Pharmingen™ APC BrdU Flow Kit	IC/FCM	200	557892
BD Pharmingen™ FITC BrdU Flow Kit	IC/FCM	50	559619
BD Pharmingen™ FITC BrdU Flow Kit	IC/FCM	200	557891
BD Pharmingen™ Cell Cycle Kit	Bioimg	50	558662

*Additional BrdU single-vial reagents in a multitude of colors are available at bdbiosciences.com.

†Bioimg=Bioimaging, IC/FCM=Intracellular Staining (Flow Cytometry)



Class 1 Laser Product.
For Research Use Only. Not for use in diagnostic or therapeutic procedures.
Alexa Fluor® is a registered trademark of Life Technologies Corporation.
Trademarks are the property of their respective owners.
23-17143-02

BD Life Sciences, San Jose, CA, 95131, USA

bdbiosciences.com