EdU Cell Proliferation Assay

EdU-488 Catalog No. 17-10525
EdU-555 Catalog No. 17-10526
EdU-594 Catalog No. 17-10527
EdU-647 Catalog No. 17-10528

100 reactions

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

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Introduction

Cell proliferation is characterized by *de novo* DNA synthesis during the S-Phase of the cell cycle. Measurement of DNA synthesis is therefore essential for research in many scientific fields, such as assessing cell health, determining genotoxicity and evaluating the impact of small molecules on cell cycle. The traditional approach for monitoring DNA synthesis and cell proliferation is to measure the incorporation of [^{3}H] thymidine as cells enter S phase. Subsequent quantification of [^{3}H] thymidine is performed by scintillation counting or autoradiography. This technology is slow, labor intensive and has several limitations including the handling and disposal of radioisotopes, as well as the need for expensive equipment.

Another well-established detection method uses bromodeoxyuridine (BrdU), a thymidine analog, followed by detection using antibodies to BrdU. For this application, BrdU is incorporated into newly synthesized DNA strands of actively proliferating cells. Following partial denaturation of double stranded DNA, incorporated BrdU is detected immunochemically by anti-BrdU antibody. The major disadvantage of the BrdU method is that BrdU antibodies will only react with single stranded DNA since double-stranded DNA blocks the access of anti-BrdU antibody to incorporated BrdU molecules. Therefore samples have to be subjected to harsh denaturing conditions which increases the time to perform the assay and can result in degradation of the structure of the specimen.

To overcome these disadvantages, EMD Millipore offers EdU (5-ethynyl-2'-deoxyuridine) cell proliferation assays. The use of EdU as a thymidine nucleoside analog is a significant improvement compared to the classical BrdU and ^{3}[H] thymidine cell proliferation assays. In contrast to BrdU assay kits, the EdU cell proliferation assays are not antibody based and therefore do not require DNA denaturation for detection of the incorporated nucleoside. Instead, our EdU Cell Proliferation assays utilize click chemistry for detection in a variety of dye fluorescent readouts. Furthermore, the streamlined detection protocol reduces the total number of steps and significantly decreases the total amount of time. It provides a faster detection procedure and is compatible with flow cytometry & microscopy.

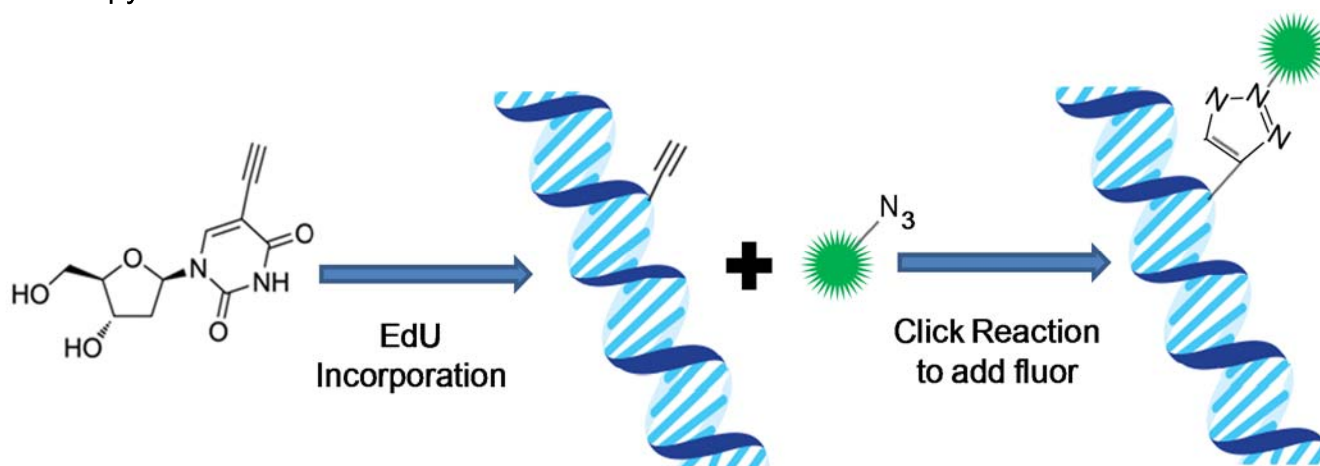

Figure 1. EdU Cell Proliferation Assay Principle. Cells grown in the presence of 5-EdU incorporate the compound at thymidine bases during S-phase. Fluorophore-labeled azide reacts with the incorporated EdU to allow detection by microscopy or flow cytometry.

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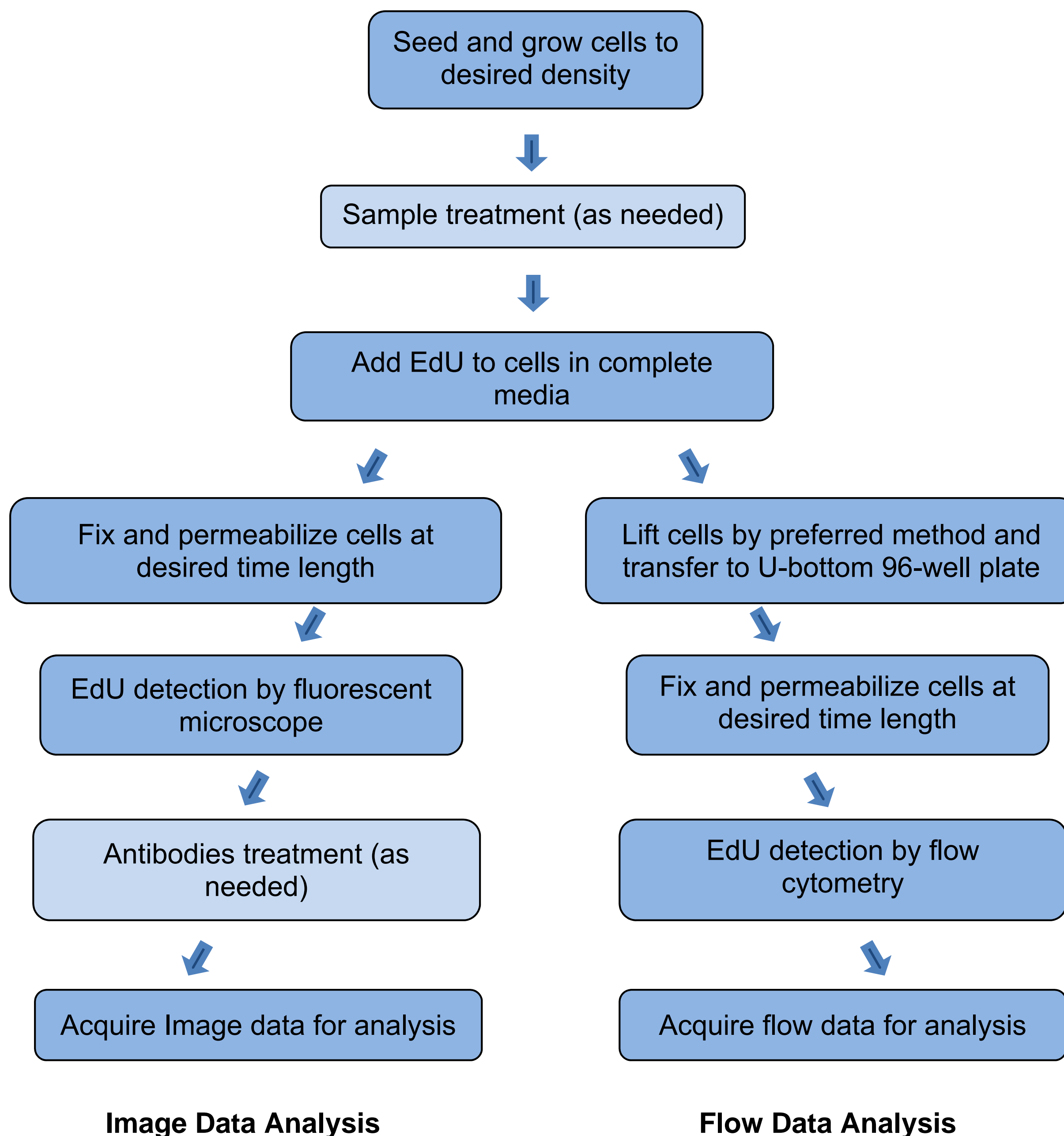

Figure 2. EdU Cell Proliferation Assay Kit Overview. Cells are grown and treated as appropriate followed by the addition of EdU to the culture media. Detection of actively proliferating cells can be performed using detection protocols for either image based or flow cytometric analysis.

Warnings and Precautions

EdU Cell Proliferation Assay Kits contain dimethyl sulfoxide (DMSO) which can penetrate skin and mucous membranes upon contact. Use gloves and other personal protective equipment when working with DMSO.

Storage and Stability

Upon receipt, store components at the temperatures indicated on the labels. Kit components are stable for 6 months from date of shipment when stored as directed.

Materials Provided (Kit Components)

The EdU cell proliferation kits include sufficient reagents for 100 reactions. Store at 2-8°C upon receipt.

Component	Part#	Amount Provided
5-Ethynyl-deoxyuridine (5-EdU)	CS219082 (component of all kits)	One 5 mg vial
Buffer additive	CS219074 (component of all kits)	Four 200 mg vials
6-FAM-Azide† (10 mM)	CS219083 (component of 17-10525 only)	One 130 µL vial
5-TAMRA-PEG3-Azide† (10 mM)	CS219051 (component of 17-10526 only)	One 130 µL vial
5/6-Sulforhodamine101-PEG3-Azide† (10 mM)	CS219039 (component of 17-10527 only)	One 130 µL vial
Eterneon-Red 645 Azide† (10 mM)	CS219040 (component of 17-10528 only)	One 130 µL vial
DMSO	CS219084 (component of all kits)	Two 2 mL vials
10X Reaction Buffer	CS219085 (component of all kits)	Four 2 mL vials
Catalyst solution	CS219038 (component of all kits)	Two 2 mL vials

† Protect fluorophore containing azide solutions from light. Stable if stored in the dark at -20° C.

Materials Required But Not Supplied

Reagents

- Cells adherently grown on a coverslip
- Phosphate-buffered saline (PBS, pH 7.2-7.6)
- Appropriate cell culture medium
- Fixation solution (3.7% formaldehyde in PBS)
- Permeabilization solution (for example, 0.5% Triton™ X-100 in PBS)
- Deionized water

Equipment

- Reaction tubes (size depends on the volume of reaction cocktail needed)
- 18 x 18 mm coverslips
- Optional: 6-well microplate

Detailed Protocol

A. Working Stock solution Preparation

Allow all vials to warm to room temperature before opening.

1. Prepare a 10 mM stock solution of EdU (Part No. CS219082): Add 2 mL of DMSO (Part No. CS219084) to the vial and mix to completely dissolve the EdU. Store any remaining solution at -20° C. When stored as directed, stock solution is stable for up to one year.
2. Prepare a 10X stock solution of the buffer additive (Part No. CS219074): Add 2 mL of deionized water to each of the vials and mix to have the compound dissolved completely. Store any remaining solution at -20° C. This stock solution is stable for up to 6 months if stored as directed. If solution starts to develop a brown color, it has degraded and should be discarded. Aliquots are recommended to avoid repeated freeze/thaw cycles.

B. EdU Incorporation, Fixation and Permeabilization

Any adherent cell type can be adapted to this procedure. It is recommended to start with a 10 µM EdU. Some factors such as cell type variation, cell density, and growth medium may influence the labeling. Procedures for microscopic imaging and flow cytometry analysis are presented below. Be sure to follow the correct procedure for your desired method.

Microscopic Imaging

I. EdU Incorporation Procedure

1. Plate cells in 200 µL suspension per well on an 8-well glass chamber slide at a desired density. Allow cells recover overnight (≈ 80% confluence).
   * Recommended chamber slide: EMD Millipore, Millicell® EZ SLIDE 8-well glass, PEZGS0816 (pack of 16), PEZGS0896 (pack of 96).
2. Prepare a 10X working solution of EdU in pre-warmed medium from the 10 mM EdU stock solution prepared in step A.1. A suggested starting concentration range is 10-20 µM final.

   For example to apply 10 µM final EdU concentration, make a 100µM EdU working solution by diluting 10mM stock 1:100 in complete media. Apply 20 µL of 100 µM working solution to each well containing of 200 µL media.
3. Incubate the cells for the desired time course under conditions optimal for the cell type.
4. Proceed immediately to fixation and permeabilization steps.

II. Cell Fixation and Permeabilization

In this protocol cells are first fixed using 3.7% formaldehyde diluted in PBS, followed by a 0.5% Triton X-100 permeabilization step.

1. Add 200 µL 3.7% formaldehyde in PBS (fixation solution) to each well containing the chamber slide. Incubate for 15 minutes at room temperature.
2. Remove the fixation solution and wash cells twice with 200 µL of 3% BSA in PBS per well.
3. Remove the wash solution and add 200 µL of 0.5% Triton X-100 in PBS (permeabilization solution) to each well. Incubate for 20 minutes at room temperature.
4. Wash the cells twice with 200 µL of 3% BSA in PBS.
5. Proceed to Fluorescent EdU Detection steps.

Flow Cytometry

I. EdU Incorporation Procedure

1. Plate cells in 100 µL suspension per well on a 96-well clear flat-bottom tissue culture plate at a desired density. Allow cells to recover overnight (≈ 80% confluence).
2. Prepare a 10X working solution of EdU in pre-warmed medium from the 10 mM EdU stock solution prepared in step A.1. A suggested starting concentration range is 10-20 µM final.

   For example to apply 10µM final EdU concentration, make a 100 µM EdU working solution by diluting 10 mM stock 1:100 in complete media. Apply 10 µL of 100 µM working solution to each well containing 100 µL media.
3. Incubate the cells for the desired time course under conditions optimal for the cell type.
4. Aspirate culture media and rinse cells once with 100 µL PBS per well.
5. Add 100 µL per well of desired reagent to detach adherent cells to generate cell suspension for flow analysis. Typically, 0.25% Trypsin-EDTA at pH 7.0 is used for this purpose. Incubate the plate in a 37°C incubator with 5% CO_2 for 15-30 minutes, check under microscope periodically to ensure all cells are in suspension.
6. Add 100 µL complete media to stop the reaction, transfer a total of 200 µL cell suspension to a new 96-well U-bottom plate. Collect cells by centrifuging with a plate adaptor at 300-400 x g for 5 minutes.
7. Discard the culture media by carefully pipetting without disturbing the cell pellets.
8. Proceed immediately to fixation and permeabilization steps.

II. Cells Fixation and Permeabilization

In this protocol cells are first fixed using 3.7% formaldehyde diluted in PBS, followed by a 0.5% Triton X-100 permeabilization step.

1. Add 100µL 3.7% formaldehyde in PBS (fixation solution) to each well of the 96-well plate. Incubate for 15 minutes at room temperature.
2. Remove the fixation solution by centrifuging the plate at 300-400 x g for 5 minutes then carefully remove solution without disturbing the cell pellets.
3. Wash the cells twice with 200 µL of 3% BSA in PBS. Centrifuge the plate at 300-400 x g for 5 minutes. Carefully remove solution without disturbing the cell pellet
4. Add 200 µL of 0.5% Triton X-100 in PBS (permeabilization solution) to each well. Incubate for 20 minutes at room temperature
5. Carefully remove the permeabilization solution after centrifuging the plate.
6. Wash cells twice with 200 µL of 3% BSA in PBS, discard wash buffer by centrifuging the plate at 300-400 x g for 5 minutes.
7. Proceed to Fluorescent EdU Detection steps.

C. Fluorescent EdU detection

For slides or 96-well plates, 100 µL of the reaction cocktail per well is recommended. Larger or smaller volumes can be applied per customer's consideration, as long as the reaction components are used in the same ratios.

1. Prepare the reaction cocktail as described in the following table. Mix the ingredients in the order listed. **Note:** Use the cocktail within 15 minutes of preparation.

Reaction Cocktail (100 µL per well):

Component	Part Number	Volume
Deionized water	-	75.8 µL
Reaction buffer (10X)	CS219085	10 µL
Catalyst solution	CS219038	4 µL
Dye-Azide (10 mM)	CS219083	0.2 µL
Buffer additive (10X) –prepared in A	CS219074	10 µL
Total Volume		**100 µL**

2. Add 100 µL of reaction cocktail to each well. Rock the slide or plate gently to distribute the reaction cocktail evenly.
3. Incubate for 30 minutes at room temperature. Protect from light!
4. Remove the reaction cocktail; wash the cells three times with 200 µL of 3% BSA in PBS. Remove the wash solution. For flow application, wash the cells three times with 200 µL of 3% BSA in PBS by centrifugation. Be careful not to disturb cell pellets when removing wash solution.

Optional: Proceed with nuclear staining (DAPI or Hoechst 33342) or antibody labeling.

Important: Protect samples from light during incubations. If no additional staining is desired, proceed with imaging and analysis.

Data Analysis

Microscopic Imaging:

EdU treated cells are compatible with all methods of slide preparation, including wet mount or prepared mounting media.

Collect images with your choice of fluorescent microscope system following the excitation and emission settings shown in table below for type of azide dye applied.

Flow Cytometry:

Resuspend cells in 200µL of 3% BSA in PBS, transfer to a V-bottom clear 96-well plate (Eppendorf® tubes can be used instead of a microplate). Pipette up and down to suspend cells to have a single cell suspension. Run samples on FACS machine and collect flow data.

Table 1: Excitation and Emission Settings

Catalog	Azide Dye	Excitation	Emission
17-10525	6-FAM	496nm	516nm
17-10526	5-TAMRA	546nm	579nm
17-10527	5/6-Sulforhodamine 101	584nm	603nm
17-10528	Eterneon-Red 645	646nm	662nm

Appendix

A. Example of Microscopic Imaging Data Using EdU Cell Proliferation Assay Kit

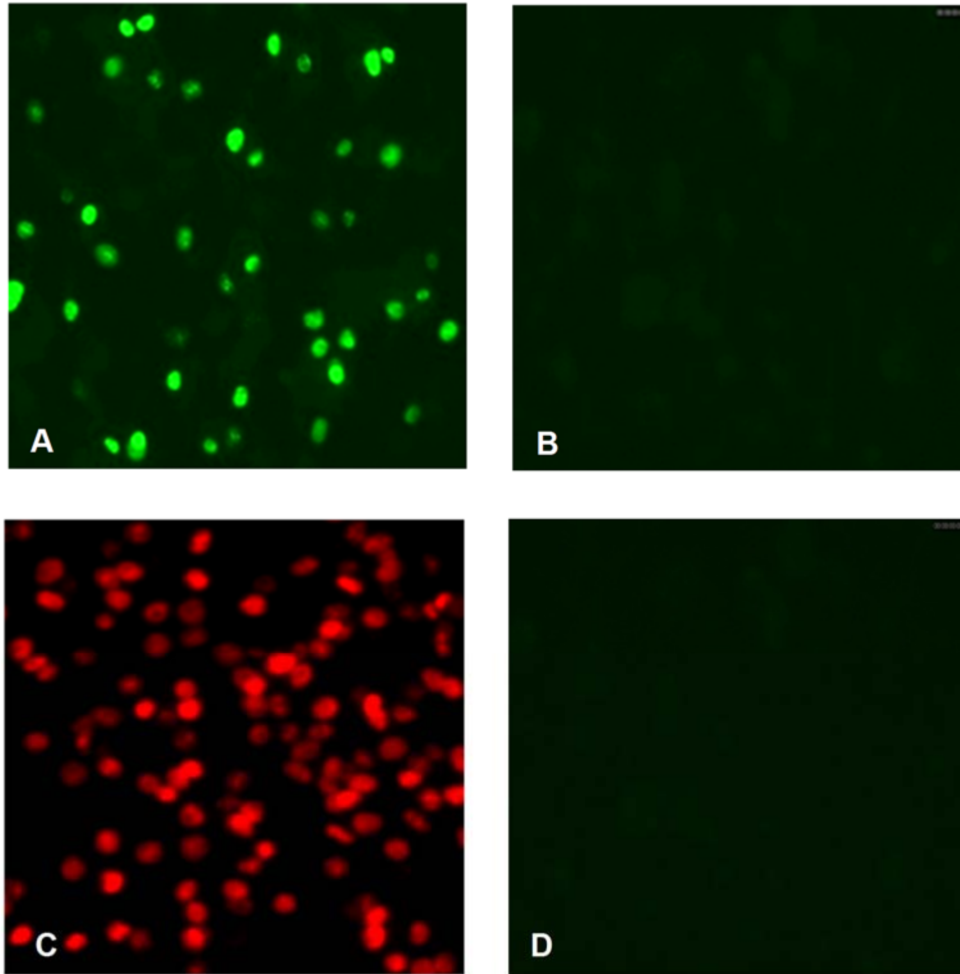

Figure 3. Cell proliferation Images monitored by EdU assay kit.
HeLa cells were grown to 70-90% confluency in a 8-well chamber slide. One set of cells were treated with 10 µM Aphidicolin (panels B and D) for four hours to cause G1/S cell cycle arrest. Other sets of cells (A and C) were not treated as a controls. For all samples the EdU cell proliferation assay was performed as described in the instruction manual using 50 µM of EdU. Cell images were processed with Leica DMI6000B fluorescence microscope. Images A and B were generated using EdU-488 (green color, catalog 17-10525). Images C and D were generated using EdU-555 (red color, catalog 17-10526).

B. Example of Flow Cytometry Using EdU cell proliferation Assay Kit

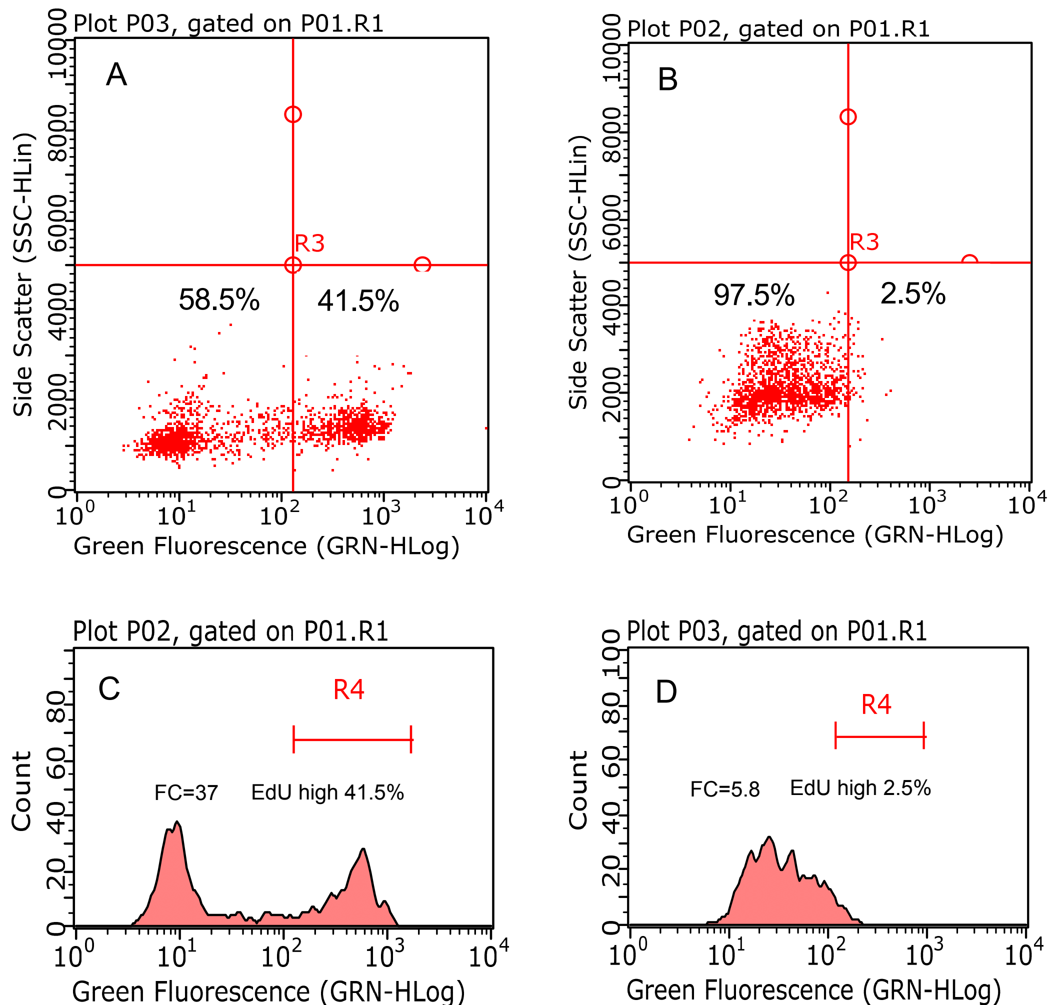

Figure 4. Flow Analysis of Cell Proliferation by EdU Assay Kit

HeLa cells were seeded in a 96-well plate and grown to 70-90% confluency. Cells were treated with or without 10μM Aphidicolin for 24 hrs to cause G1/S cell cycle arrest, followed by incubation with 10μM EdU for an additional 4 hrs. Flow cytometry data were collected and analyzed according to the flow procedure outlined in the instruction manual. For the data shown above, EdU-488 (cat.17-10525) was used. The cell population was gated for EdU-488 fluorescence intensity to indicate normal cell proliferation with untreated cells (panels A and C) or cells treated with 10μM Aphidicolin (panels B and D).

Panels A and C: normal cell proliferation with balanced population, show 58.5% non-proliferating and 41.5% highly proliferating cells (panel A). Fold change (FC) of high over low proliferation is 37 (panel C). Panels B and D: blocked cell proliferation show low EdU-488 fluorescence intensity, 97.5% non-proliferating and 2.5% proliferating cells (panel B). FC of high over low proliferation is 5.8 (panel D).

Additional cell types including A549, MDA-MB-231 and U2OS were tested by flow analysis. All cell lines examined demonstrated a similar pattern as HeLa cells. Data not shown

EdU Cell Proliferation Assay Optimization and Troubleshooting

Potential Problems	Experimental Suggestions
Fluorescent signal too weak to be visualized with microscope	• Titrate EdU with each cell model to optimize concentration. • Configure the fluorescent microscope to ensure the optimal filter is applied per each dye. • Be sure to carefully follow the assay instructions and check the buffer additive color (discard if turning brown).
Low signal for flow data or cell loss during process	• Monitor cells under microscope to make sure all cells are resuspended and collected. • Prevent cell loss during assay procedure by centrifuging down at 300-400xg for 5 minutes every time changing solutions. • Take extra care when discarding supernatant to be sure to not disturb the cell pellet. Cell pellets may not be visible to naked eye.

Guidance for EdU Concentrations in Different Cell and Tissue Types

Cell Line/Organism	Final 5-EdU concentration
HeLa cells	10 µM
CEM cells	20 µM
BT474 cells	0.1-20 µM
Jurkat cells	10 µM
NIH3T3 cells	10 µM
SK-BR-3 cells	0.1-10 µM
Primary human fibroblast	10 µM
Mouse embryo	10-200 mg/kg
Chicken embryo	500 µM
Primary valvular interstitial cells	10 µM
Drosophila (neuroblasts, salivary glands, wing discs)	15 µM
Plants (Alfalfa, Arabidopsis, grape, maize, rice tobacco)	10 µM

Related Products

Product	Description	Cat. Number
Apo-BrdU™ Detection Kit, 50 assays	Two color staining method for labeling DNA breaks and total cellular DNA to detect apoptotic cells by flow cytometry.	APT115
Apo-BrdU™ IHC Kit, 50 assays	Two color staining method for labeling DNA breaks and total cellular DNA to detect apoptotic cells by immunohistochemistry	APT116
BrdU Cell Proliferation Kit 200 assays	Assess cell proliferation by BrdU-mouse monoclonal antibody	2750
BrdU Cell Proliferation Kit 1000 assays	Assess cell proliferation by BrdU-mouse monoclonal antibody	2752
BrdU IHC Kit, 50 assays	Non-isotopic immunohistochemistry staining	2760

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