

## Product datasheet

# EdU Assay / EdU Staining Proliferation Kit (iFluor 647) ab222421

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### Overview

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<b>Product name</b>	EdU Assay / EdU Staining Proliferation Kit (iFluor 647)
<b>Detection method</b>	Fluorescent
<b>Sample type</b>	Adherent cells, Suspension cells
<b>Assay type</b>	Cell-based
<b>Product overview</b>	EdU Assay / EdU Staining Proliferation Kit (iFluor 647) ab222421 provides a sensitive and robust method to detect and quantify cell proliferation in live mammalian cells using flow cytometry or fluorescence microscopy. The iFluor 647 dye (Ex/Em: 649/664 nm) has spectral properties almost identical to those of Cy5 <sup>®</sup> and alternative red fluorophores.

EdU staining protocol summary (wash cells between each step):

- add EdU solution to cells to be stained
- incubate cells for 2-4 hrs under optimal growth conditions
- add fixative solution and incubate for 15 min
- add permeabilization buffer and incubate for 15/20 min
- add reaction mix to fluorescently label EdU and incubate for 30 min
- analyze with flow cytometer / fluorescence microscope

EdU staining can also be combined with antibody staining or cell staining with other fluorescent dyes.

This kit provides enough reagents to perform 50 flow cytometry tests or 50 microscopy tests (for 18 x 18 mm coverslips) or 200 microscopy tests (adapted for 96-well plate format).

<b>Notes</b>	<p>Previously called EdU Proliferation Assay Kit (iFluor 647).</p> <p>The most accurate method to measure DNA proliferation is by directly measuring DNA synthesis. The most common method for this uses antibody-based detection of the nucleoside analog bromo-deoxyuridine (BrdU).</p> <p>EdU (5-ethynyl-2'-deoxyuridine), a thymidine analog that is an alternative to BrdU, is also used in DNA proliferation assays that are simpler and faster than the BrdU assay. NB: EdU is also available as free molecule as <a href="#">ab146186</a> (EdU).</p> <p>In EdU staining, EdU is incorporated into newly synthesized DNA by cells within a sample. A fluorescent azide, such as iFluor-488, is then added. The fluorescent azide is small enough to</p>
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diffuse freely through native tissues and DNA, and it covalently cross-links to the EdU in a 'click' chemistry reaction.

The main advantages of EdU staining over using BrdU are:

- no harsh DNA hydrolysis / DNA denaturing step is required with EdU staining (unlike in the BrdU assay where it is used to give the BrdU antibody access to BrdU within the DNA)
- EdU staining is faster, and has less steps, than BrdU staining

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## Platform

Flow cytometer, Fluorescence microscope

## Properties

### Storage instructions

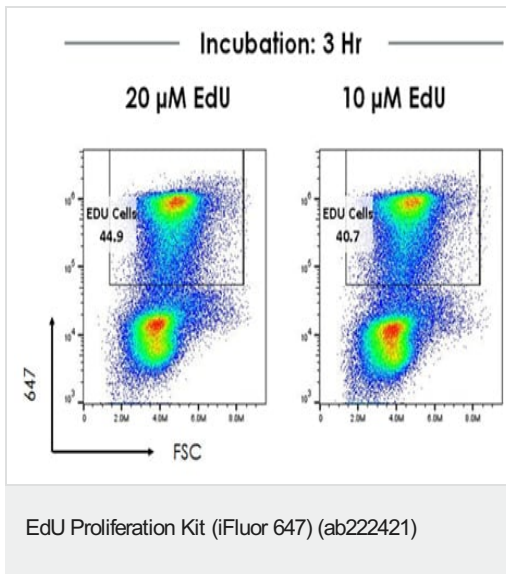
Store at -20°C. Please refer to protocols.

Components	50 tests
10X Permeabilization Buffer	1 x 25ml
Azide iFluor 647 Dye (500 µM)	1 x 130µl
Copper Sulfate (100 mM)	1 x 1ml
Dimethylsulfoxide (DMSO)	1 x 4.25ml
EdU	1 x 10mg
Fixative (40% formaldehyde solution)	1 x 5ml
Sodium Ascorbate	1 x 400mg

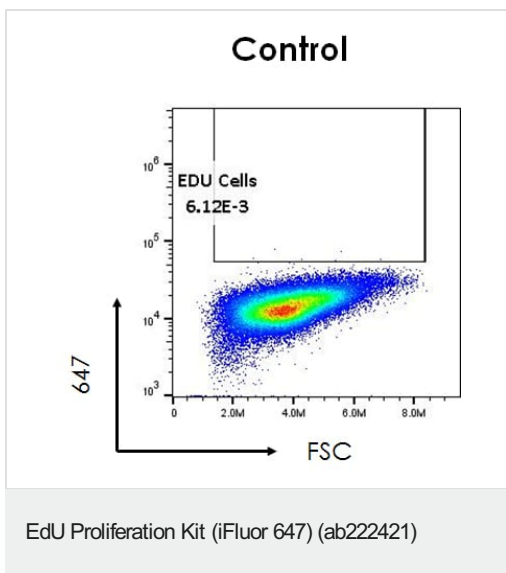
## Relevance

Cell proliferation is the multiplication or reproduction of cells, as a result of cell growth and cell division, resulting in the expansion of a cell population.

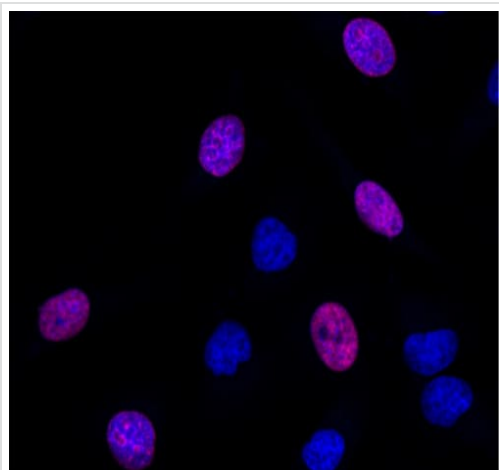
## Images



Dot plot of EdU-647 staining (Y-axis, 647) vs FSC.  $10^6$  HeLa cells were incubated with the stated concentrations of EdU for 3 hours. Control cells (next image) were incubated with media only. Images were acquired on an Accuri C6 Cytometer (BD Biosciences) with cells excited using a 640 nm laser and data analyzed using FlowJo (v10). The percentage of gated cells (EdU positive) is highlighted.

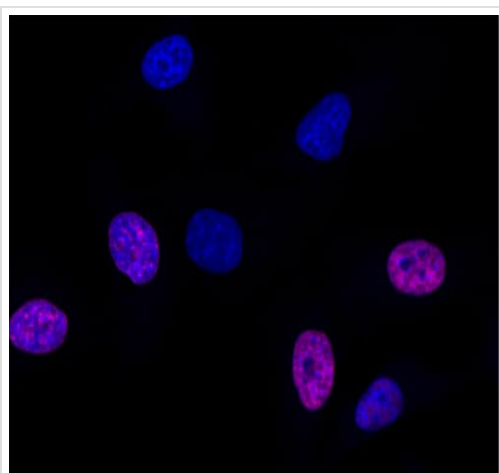


Dot plot of EdU-647 staining (Y-axis, 647) vs FSC.  $10^6$  HeLa cells were incubated with the stated concentrations of EdU for 3 hours. This image shows control cells, incubated with media only. Images were acquired on an Accuri C6 Cytometer (BD Biosciences) with cells excited using a 640 nm laser and data analyzed using FlowJo (v10). The percentage of gated cells (EdU positive) is highlighted.



EdU Proliferation Kit (iFluor 647) (ab222421)

EdU staining of proliferating cells. HeLa cells ( $4 \times 10^4$  cells/well in 96 plate) were incubated with 20  $\mu$ M EdU for 3 hours. Cells were analyzed using a TCS SP8 confocal microscope (Leica-Microsystems). DNA (blue) was staining with **Hoechst 33342** (**ab145597**). Purple cells show EdU/Hoechst-positive cells.



EdU Proliferation Kit (iFluor 647) (ab222421)

EdU staining of proliferating cells. HeLa cells ( $4 \times 10^4$  cells/well in 96 plate) were incubated with 10  $\mu$ M EdU for 3 hours. Cells were analyzed using a TCS SP8 confocal microscope (Leica-Microsystems). DNA (blue) was staining with **Hoechst 33342** (**ab145597**). Purple cells show EdU/Hoechst-positive cells.

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