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Product datasheet

EdU Assay / EdU Staining Proliferation Kit (iFluor 488) ab219801

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Overview

Product name EdU Assay / EdU Staining Proliferation Kit (iFluor 488)

Detection method Fluorescent

Sample type Adherent cells, Suspension cells

Assay type Cell-based

Product overview EdU Assay / EdU Staining Proliferation Kit (iFluor 488) ab219801 provides a sensitive and

robust method to detect and quantify cell proliferation in live mammalian cells using flow cytometry or fluorescence microscopy. The iFluor 488 dye (Ex/Em: 491/520 nm) has spectral properties

almost identical to those of FITC and alternative green 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).

Previously called EdU Proliferation Assay Kit (iFluor 488).

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).

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 **ab146186** (EdU).

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

Notes

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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

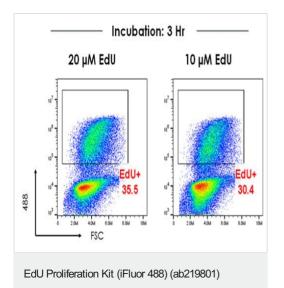
Properties

Storage instructions

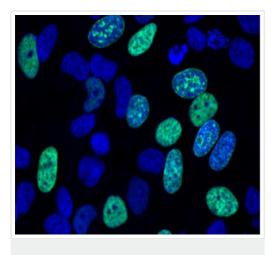
Store at -20°C. Please refer to protocols.

Components	50 tests	50 tests
10X Permeabilization Buffer	1 x 25ml	1 x 25ml
Copper Sulfate (100 mM)	1 x 1ml	1 x 1ml
Dimethylsulfoxide (DMSO)	1 x 4.25ml	1 x 4.25ml
EdU	1 x 10mg	1 x 10mg
Fixative (40% formaldehyde solution)	1 x 5ml	1 x 5ml
iFluor 488 azide dye (500 μM)	1 x 130µl	1 x 130µl
Sodium Ascorbate	1 x 400mg	1 x 400mg

Images



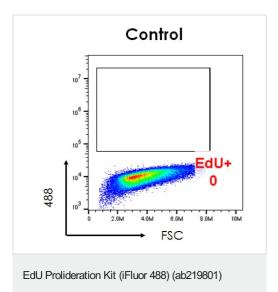
Dot plot of EdU-488 staining (Y-axis, 488) vs FSC. 10⁶ HeLa (Human epithelial cell line from cervix adenocarcinoma) cells were incubated with the stated concentrations of EdU for 3 hours. Control cells (next image) were incubated with media only. Images were acquired on an Accuri C6 Cytometer (BD Biosciences) with cells excited using a 488 nm laser and data analyzed using FlowJo (v10). The percentage of gated cells (EdU positive) is highlighted.



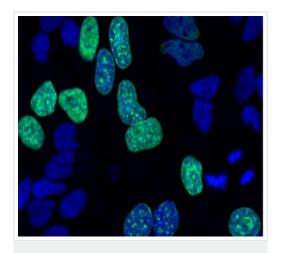
from cervix adenocarcinoma) cells (4 x 10^4 cells/well in 96 plate) were incubated with 20 μ M EdU for 3 hours. Cells were analyzed using a TCS SP8 confocal microscope (Leica-Microsystems). DNA (blue) was staining with Hoechst 33342 **ab145597**. Green cells show EdU/Hoechst-positive cells.

EdU staining of proliferating cells. HeLa (Human epithelial cell line

EdU Proliferation Kit (iFluor 488) (ab219801)



Dot plot of EdU-488 staining (Y-axis, 488) vs FSC. 10⁶ HeLa (Human epithelial cell line from cervix adenocarcinoma) 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 488 nm laser and data analyzed using FlowJo (v10). The percentage of gated cells (EdU positive) is highlighted.



EdU Proliferation Kit (iFluor 488) (ab219801)

EdU staining of proliferating cells. HeLa (Human epithelial cell line from cervix adenocarcinoma) cells (4 x 10^4 cells/well in 96 plate) were incubated with 10 μ M EdU for 3 hours. Cells were analyzed using a TCS SP8 confocal microscope (Leica-Microsystems). DNA (blue) was staining with Hoechst 33342 **ab145597**. Green cells show EdU/Hoechst-positive cells.

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