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

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).

Notes

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-ethyl-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.

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 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

**Platform**
Flow cytometer, Fluorescence microscope

**Properties**

**Storage instructions**
Store at -20°C. Please refer to protocols.

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

**Images**

Dot plot of EdU-488 staining (Y-axis, 488) vs FSC. 10^6 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.
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 20 μM 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.

Dot plot of EdU-488 staining (Y-axis, 488) vs FSC. 10^6 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 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 μM 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.
Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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