Bucculite™ Flow Cytometric XdU Cell Proliferation Assay Kit *Red Laser-Comptatible*

Catalog number: 22325
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 22325)
Component A: XdU	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: iFluor® 647-MTA	Freeze (< -15 °C), Minimize light exposure	1 vial
Component C: Staining Buffer	Freeze (< -15 °C), Minimize light exposure	1 bottle (20 mL)
Component D: 10X Washing Buffer	Freeze (< -15 °C)	1 bottle (10 mL)
Component E: DMSO	Freeze (< -15 °C)	1 vial (1 mL)
Component F: Hoechst 33342	Freeze (< -15 °C), Minimize light exposure	1 vial (50 uL, 10 mg/mL in water)

OVERVIEW

Monitoring cell proliferation is one of the most reliable methods to assess cell viability, cell cycles and genotoxicity. An essential way to detect cell proliferation is to measure DNA synthesis in the presence of thymidine during the S-phase of cells growth. Bucculite™ Flow Cytometric XdU Cell Proliferation Assay Kit uses XdU which is incorporated into cellular DNA during DNA synthesis. After fixing cells, the incorporated XdU is labelled with iFluor® 647 MTA. The resulted iFluor® 647-labeled DNA formed in cells is visualized in Cy5 Channel. Bucculite™ Flow Cytometric XdU Cell Proliferation Assay Kit provides an alternative to anti-BrdU antibody-based assay and EdU click chemistry assay. It is sensitive and might be used for measuring active DNA synthesis at single-cell level.

AT A GLANCE

Protocol Summary

1. Prepare cells (100 µL/well for a 96-well plate or 25 µL/well for a 384-well plate)
2. Add 2X XdU working solution 100 µL/well for a 96-well plate
3. Incubate at 37 °C for 3 hours
4. Remove the media and fix cells with 100 µL ice cold 90% Methanol in PBS for 15 minutes at room temperature
5. Remove Fixation buffer and wash three times with PBS
6. Add 1X iFluor™ 647-MTA working solution (200 µL/tube) and stain for 30 mins at room temperature
7. Remove working solution in each tube and wash cells with 1X Washing Buffer three times
8. Add 100 µL 1X Washing Buffer /tube and observe with flow cytometry using 660/20 nm filter

KEY PARAMETERS

Flow cytometer

Emission	660/20 nm filter
Excitation	633/640 nm laser
Instrument specification(s)	APC channel

CELL PREPARATION

For guidelines on cell sample preparation, please visit https://www.aatbio.com/resources/guides/cell-sample-preparation.html

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

XdU stock solution (1000X)

Add 500 µL DMSO (Component E) into XdU (Component A) to make 1000X stock solution. **Note:** This 1000X concentration was developed with HeLa cells with an optimized XdU concentration. Growth medium, cell density, cell type variations, and other factors may influence the labeling. We recommend testing a range of FOL-FdU concentrations to determine the optimal concentration for your cell type and experimental conditions.

iFluor™ 647-MTA stock solution (400X)

Add 50 µL of DMSO (Component E) to iFluor™ 647-MTA (Component B) to make 400 X iFluor™ 647-MTA stock solution

PREPARATION OF WORKING SOLUTION

XdU working solution (2X)

Dilute 1000X XdU stock solution by 500 folds in complete medium to prepare a 2X XdU working solution.

iFluor™ 647-MTA working solution (1X)

Add 2.5 µL 400X iFluor™ 647-MTA stock solution to 1 mL Staining Buffer (Component C) to prepare 1X iFluor™ 647-MTA working solution.

Washing Buffer (1X)

Add 1 mL 10X washing buffer (Component D) to 9 mL PBS to make 1X Washing Buffer.

SAMPLE EXPERIMENTAL PROTOCOL

Prepare Cells

1. For adherent cells: Plate cells overnight in growth medium at 10,000 to 40,000 cells/well/100 µL for a 96-well plate or 2,500 to 10,000 cells/well/20 µL for a 384-well plate.
2. For non-adherent cells: Centrifuge the cells from the culture medium and suspend the cell pellets in culture medium at 1-2 X 10^6 cells/ml (10 mL for one 96-well plate). **Note:** Each cell line should be evaluated on an individual basis to determine the optimal cell density.

Labeling Cells with XdU

1. Add an equal volume of the 2X XdU working solution to the volume of media containing cells to be treated to obtain a 1X XdU solution in each well. We do not recommend replacing all of

the media, because this could affect the rate of cell proliferation.

2. Incubate the cells for the 3 hours under conditions optimal for the cell type. The time of XdU exposure to the cells allows for direct measurement of cells synthesizing DNA. The incubation time depends on the cell growth rate.

Cell Fixation

1. After incubation, remove the media and add 100 µL ice cold 90% Methanol in PBS (not provided, Methanol/PBS, v/v is 90/10) to each well, and incubate for 15 minutes at room temperature. **Note:** After washing step, if using attached cells, then detach them using ReadiUse™ Cell Detaching Buffer (AAT Cat# 60010).

2. Remove the fixation buffer and wash the cells in each tube twice with PBS.

Stain Cells

1. Add 200 µL/tube of 1X iFluor™ 647-MTA working solution in the cell plate. Incubate cells with working solution at room temperature for 30 minutes, protected from light.

2. Remove working solution in each tube.

3. Wash cells with 1X Washing Buffer three times, and add 100 µL Washing Buffer /tube after wash. **Note:** If Hoechst 33342 stain is needed, make 5-10 µg/mL Hoechst 33342 solution in 1X Washing Buffer and stain for 30 mins.

4. Observe the fluorescence signal in cells using flow cytomtry with a 660/20 nm filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

Placeholder for image details

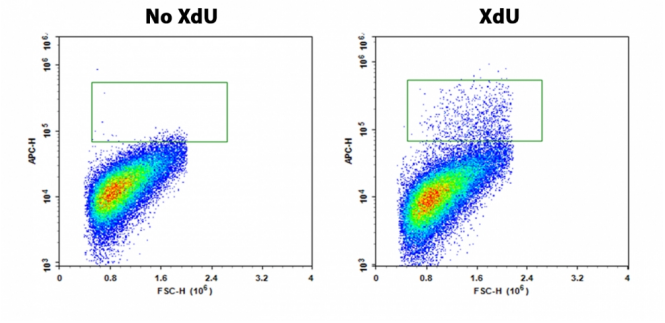

Figure 1. S-phase Jurkat cells were detected with Bucculite™ XdU Cell Proliferation Flow Cytometry Kit (Cat#22325). Jurkat cells at 50,000 cells/well/100 µL were seeded overnight in a 96-well black wall/clear bottom plate. Cells were treated with XdU at 37 °C for 3 hours, and fixed with Methanol/PBS (90/10). After fixation, cells were stained with iFluor® 647-MTA for 30 min in staining buffer, and then washed three times with 1X washing Buffer. 100 µL 5 µg/ml Hoechst 33342 solution in 1X Washing Buffer were added to each well and the flow cytometry was performed using flow cytometry with APC channel.

DISCLAIMER

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