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 azide	Freeze (< -15 °C), Minimize light exposure	1 vial
Component C: Staining Buffer	Freeze (< -15 °C), Minimize light exposure	1 bottle (20 mL)
Component D: CuSO4	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mL)
Component E: XdU reaction additive	Freeze (< -15 °C), Minimize light exposure	1 vial (400 mg)
Component F: Hoechst 33342	Freeze (< -15 °C), Minimize light exposure	50 µL (10 mg/mL in water)
Component G: DMSO	Freeze (< -15 °C), Minimize light exposure	400 µL

OVERVIEW

Bucculite™ Flow Cytometric XdU Cell Proliferation Assay Kit provides a fast and mild method for analyzing active DNA synthesis in proliferating cells by flow cytometry. The assay detects XdU incorporation into newly synthesized DNA, enabling direct measurement of cells undergoing S-phase progression without the harsh DNA denaturation steps required for traditional BrdU-based detection. The kit is designed for multicolor flow cytometry workflows and uses chemistry developed to minimize copper-related quenching of fluorescent proteins and phycobiliprotein-based fluorophores such as R-PE and PE-tandems. Its streamlined workflow avoids lengthy antibody incubation steps and supports completion of labeling and detection in as little as 60 minutes. Bucculite™ Flow Cytometric XdU Cell Proliferation Assay Kit is suitable for cell proliferation analysis, S-phase quantification, compound response studies, and multiplex flow cytometry applications.

AT A GLANCE

Protocol Summary

1. Prepare cells
2. Treat cells with the XdU working solution for 3 hours
3. Fix cells
4. Permeabilize cells
5. Treat cells with the Bucculite™ working solution for 30 minutes
6. Remove the working solution and image cells using flow cytometry with a 660/20 filter set

KEY PARAMETERS

Flow cytometer

Excitation	633 or 640 nm laser
Emission	660/20 nm filter
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 (100X)
Dissolve XdU (Component A) in 200 µL of DMSO (Component G) to make XdU stock solution. **Note:** The remaining XdU stock solution can be stored in smaller aliquots at -20°C. Protect from light and avoid repeated freeze-thaw cycles.

iFluor® 647-azide stock solution (200 X)
Dissolve iFluor® 647-azide (Component B) in 100 µL of DMSO (Component G) to make an iFluor® 647-azide stock solution. **Note:** The remaining stock solution can be stored in smaller aliquots at -20 °C. Protect from light and avoid repeated freeze-thaw cycles

XdU reaction additive stock solution (20X)
Dissolve XdU reaction additive (Component E) in 1 mL of deionized water to make a XdU reaction additive stock solution. **Note:** The remaining XdU reaction additive stock solution can be stored at 2-8 °C.

PREPARATION OF WORKING SOLUTION

XdU working solution
Add 10 µL of the 100X XdU stock solutions in 1 mL of the cell culture medium to make XdU working solution. **Note:** 1 mL of the working solution is enough for 5 tests. This 100X concentration was developed with HeLa and Jurkat cells using an optimized XdU concentration. Growth medium, cell density, cell type variations, and other factors may influence the labeling. We recommend testing a range of XdU concentrations to determine the optimal concentration for your cell type and experimental conditions.

Bucculite™ working solution
Mix in the following order and vortex well.
905 µL of Staining Buffer (Component C)
40 µL of CuSO4 (Component D)
5 µL of iFluor® 647-azide
50 µL of the XdU reaction additive stock solution
Note: 1 mL of the Bucculite™ working solution is enough for 5 tests.

SAMPLE EXPERIMENTAL PROTOCOL

Cells labelling with XdU

1. Add 200 µL of XdU working solution to the cells considering the cells are in 200 µL cell culture medium.
2. Incubate the cells for 3 hours under optimal conditions for the cell type. The time of XdU exposure to the cells allows for the direct measurement of cell DNA synthesis. The incubation time depends

on the cell growth rate.

Cells fixation:

1. After incubation, remove the media and wash cells once with PBS. **Note:** After washing step, if using attached cells, then detach them using ReadiUse™ Cell Detaching Buffer (AAT Cat# 60010).
2. Add 200 µL of fixation buffer (3.7% formaldehyde in PBS, not provided) to each tube, and incubate for 15-20 minutes at room temperature.
3. Remove the fixative and wash the cells in each tube twice with PBS.

Cells permeabilization:

1. Add 200 µL of permeabilization buffer (0.5% Triton X-100 in PBS, not provided) to each tube, and incubate for 10-20 minutes at room temperature.
2. Remove the permeabilization buffer and wash the cells in each tube twice with PBS.

Cells staining:

1. Add 200 µL Bucculite™ working solution in the tube. Incubate cells with the Bucculite™ working solution at room temperature for 30 minutes, protected from light.
2. Remove the Bucculite™ working solution in each tube.
3. Wash cells twice with PBS, and add 200 µL of PBS after wash. **Note:** If Hoechst 33342 stain is needed, make 5-10 µg/mL Hoechst 33342 solution in 1X PBS and stain for 30 mins.
4. Observe the fluorescence signal in cells using a flow cytometer using 450/40 nm filter.
5. Observe the fluorescence signal in the cells using a flow cytometer with a 660/20 filter.

EXAMPLE DATA ANALYSIS AND FIGURES

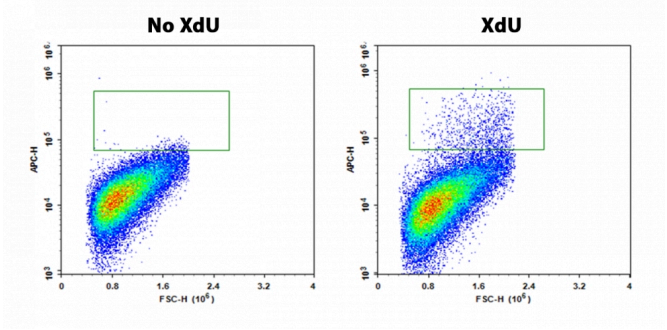

Figure 1. S-phase Jurkat cells were detected with Bucculite™ XdU Cell Proliferation Flow Cytometry Kit (Cat#22325). Jurkat cells at 50,000 cells/well/100 µL were seeded overnight in a 96-well black wall/clear bottom plate. Cells were treated with XdU at 37 °C for 3 hours, and fixed with Methanol/PBS (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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