

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

 Catalog number: 22323  
 Unit size: 100 Tests

| Component                       | Storage                                    | Amount (Cat No. 22323)            |
|---------------------------------|--|-----------------------------------|
| Component A: XdU                | Freeze (< -15 °C), Minimize light exposure | 1 vial                            |
| Component B: iFluor® 488-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® 488 MTA. The resulted iFluor® 488-labeled DNA formed in cells is visualized in FITC 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™ 488-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 using flow cytometry with 530/30 nm filter

**KEY PARAMETERS**
**Flow cytometer**

|                             |                  |
|-----------------------------|------------------|
| Emission                    | 530/30 nm filter |
| Excitation                  | 488 nm laser     |
| Instrument specification(s) | FITC 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™ 488-MTA stock solution (400X)**

Add 50 µL of DMSO (Component E) to iFluor™ 488-MTA (Component B) to make 400 X iFluor™ 488-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™ 488-MTA working solution (1X)**

Add 2.5 µL 400X iFluor™ 488-MTA stock solution to 1 mL Staining Buffer (Component C) to prepare 1X iFluor™ 488-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<sup>6</sup> 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.

- 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

- After incubation, remove the media and add 100  $\mu$ L 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).

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

### Stain Cells

- Add 200  $\mu$ L/tube of 1X iFluor™ 488-MTA working solution in the cell plate. Incubate cells with working solution at room temperature for 30 minutes, protected from light.

- Remove working solution in each tube.

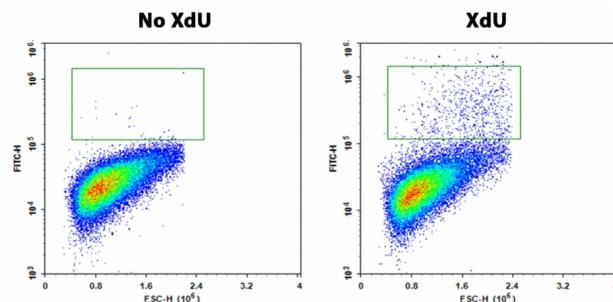
- Wash cells with 1X Washing Buffer three times, and add 100  $\mu$ L Washing Buffer /tube after wash.

**Note:** If Hoechst 33342 stain is needed, make 5-10  $\mu$ g/mL Hoechst 33342 solution in 1X Washing Buffer and stain for 30 mins.

- Observe the fluorescence signal in cells using fluorescence microscope with a FITC filter set.

Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.

### EXAMPLE DATA ANALYSIS AND FIGURES



**Figure 1.** S-phase Jurkat cells were detected with Bucculite™ XdU Cell Proliferation Flow Cytometry Imaging Kit (Cat#22323). Jurkat cells at 50,000 cells/well/100  $\mu$ L 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® 488-MTA for 30 min in staining buffer, and then washed three times with 1X washing Buffer. 100 $\mu$ L 5  $\mu$ g/ml Hoechst 33342 solution in 1X Washing Buffer were added to each well and analysed with FITC channel using flow cytometry for S phase cells.

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