Bucculite™ XdU Cell Proliferation Fluorescence Imaging Kit *Green Fluorescence*

Catalog number: 22326
Unit size: 200 Tests

Component	Storage	Amount
Component A: XdU	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: iFluor™ 488-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

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™ XdU Cell Proliferation Fluorescence Imaging Kit uses XdU which is incorporated into cellular DNA during DNA synthesis. After fixing cells, the incorporated XdU is labelled with iFluor™ 488 azide. The resulted iFluor™ 488-labeled DNA formed in cells is imaged with a FITC filter set. Bucculite™ XdU Cell Proliferation Fluorescence Imaging Kit provides an alternative to anti-BrdU antibody-based assay and EdU click chemistry-based 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
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 a fluorescence microscope with a FITC filter set

KEY PARAMETERS

Fluorescence microscope

Excitation	FITC filter set
Emission	FITC filter set
Recommended plate	Black wall/clear bottom

CELL PREPARATION

For adherent cells
Plate cells overnight in the 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.

For non-adherent cells
Centrifuge the cells from the culture medium and suspend the cell pellets in the 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.

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.

1. XdU stock solution (100X)
Dissolve XdU (Component A) in 200 µL of DMSO to make a XdU stock solution.

Note The remaining XdU stock solution can be stored in smaller aliquots at -20 °C.

Note Protect from light and avoid repeated freeze-thaw cycles.

2. iFluor™ 488-azide stock solution (200 X)
Dissolve iFluor™ 488-azide (Component B) in 100 µL of DMSO to make an iFluor™ 488-azide stock solution.

Note The remaining iFluor™ 488-azide stock solution can be stored in smaller aliquots at -20 °C.

Note Protect from light and avoid repeated freeze-thaw cycles.

3. 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 -20 °C.

PREPARATION OF WORKING SOLUTION

1. XdU working solution
Add 10 µL of the 100X XdU stock solution in 1 mL of the cell culture medium to make a XdU working solution.

Note 1 mL of the working solution is enough for 10 tests.

Note This 100X concentration was developed with HeLa 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.

2. Bucculite™ working solution
Mix in the following order and vortex well.
905 µL of Staining Buffer (Component C)
40 µL of $CuSO_4$ (Component D)
5 µL of iFluor™ 488-azide stock solution
50 µL of the XdU reaction additive stock solution

Note 1 mL of the Bucculite™ working solution is enough for 10 tests.

SAMPLE EXPERIMENTAL PROTOCOL

Cells labelling with XdU

1. Add an equal volume of the XdU working solution to the cells.

© 2020 AAT Bioquest, Inc. Last revised May 2021. For more information and tools, please visit https://www.aatbio.com

2. Incubate the cells for the 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.
2. Add 100 µL of Fixation Buffer (3.7% formaldehyde in PBS, not provided) to each well, and incubate for 15-20 minutes at room temperature.
3. Remove the fixative and wash the cells in each well twice with PBS.

Cells permeabilization

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

Cells staining

1. Add 100 µL/well (a 96-well plate) or 50 µL/well (a 384-well plate) Bucculite™ working solution in the cell plate. Incubate cells with the working solution at room temperature for 30 minutes, protected from light.
2. Remove the Bucculite™ working solution in each well.
3. Wash cells twice with PBS, and add 100µL of PBS after wash.

   Note If Hoechst 33342 stain (Component F) is needed, make 5-10 µg/mL Hoechst 33342 solution in 1X PBS and stain for 30 min.
4. Observe the fluorescence signal in cells using a fluorescence microscope with a FITC filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

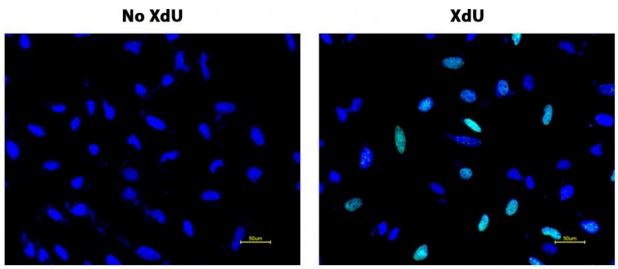

Figure 1. The images of S-phase HeLa cells detected with Buccutite™ XdU Cell Proliferation Fluorescence Imaging Kit (Cat#22326) **.** HeLa cells were seeded at 50,000 cells/well/100 µL overnight in a 96-well black wall/clear bottom plate. Cells were treated with XdU at 37 ºC for 3 hours, fixed and permeabilized as per protocol. Cells were then stained with iFluor™ 488-azide for 30 mins in staining buffer, and washed three times with PBS. 100µL of 5 µg/mL Hoechst solutions in PBS were added to each well and the fluorescence images were visualized with a FITC filter set for S phase cells (Green) and a DAPI filter set for all cell nuclei (Blue).

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.