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

Catalog number: 22327
Unit size: 200 Tests

Component	Storage	Amount (Cat No. 22327)
Component A: XdU	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: iFluor® 555 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™ XdU Cell Proliferation Fluorescence Imaging Kit provides a fast and mild method for analyzing active DNA synthesis in proliferating cells by fluorescence microscopy. The assay detects XdU incorporation into newly synthesized DNA, enabling visualization of cells undergoing S-phase progression without the harsh DNA denaturation steps required for traditional BrdU-based detection. The kit is designed for fluorescence imaging workflows and uses chemistry developed to minimize copper-related quenching of fluorescent proteins and phycobiliprotein-based fluorophores. The red fluorescent signal is compatible with standard Texas Red-compatible filter settings, making it suitable for common fluorescence microscopy platforms. The streamlined workflow avoids lengthy antibody incubation steps and supports completion of labeling and detection in as little as 60 minutes. Bucculite™ XdU Cell Proliferation Fluorescence Imaging Kit is suitable for cell proliferation imaging, S-phase analysis, compound response studies, and microscopy-based cell biology 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 with Cy3 filter set

KEY PARAMETERS

Fluorescence microscope

Excitation	Cy3 filter set
Emission	Cy3 filter set
Recommended plate	Black wall/clear bottom

CELL PREPARATION

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.

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.

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® 555-azide stock solution
Dissolve iFluor® 555-azide (Component B) in 100 µL of DMSO (Component G) to make an iFluor® 555 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:** 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 10 tests. 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.

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® 555-azide
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

Cell labelling 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.

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.
2. Add 100 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 100 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 100 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.
3. Wash cells twice with PBS, and add 100 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 fluorescence microscope with a Cy3 filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

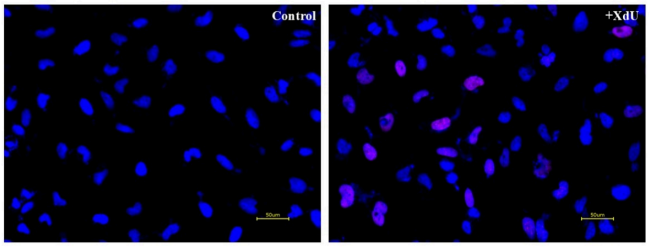

Figure 1. S-phase HeLa cells detection using the Bucculite™ XdU Cell Proliferation Fluorescence Imaging Kit. HeLa cells (50,000 cells/well in 100 µL) were seeded overnight in a 96-well black wall/clear bottom plate. Cells were treated with XdU at 37°C for 3 hours, then fixed with Methanol/PBS (90/10). After fixation, cells were stained with iFluor® 555-MTA for 30 minutes in staining buffer, followed by three washes with 1X Washing Buffer. Nuclear staining was performed with 100 µL of 5 µg/mL Hoechst 33342 solution in 1X Washing Buffer. Fluorescence images were acquired using a TRITC filter to visualize S-phase cells (red) and a DAPI filter for nuclear staining (blue).

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