AAT Bioquest®

PRODUCT INFORMATION SHEET

Bucculite™ FdU Cu-Free Cell Proliferation Fluorescence Imaging Kit *Red Fluorescence*

Catalog number: 22315
Unit size: 200 Tests

Component	Storage	Amount
Component A: FOL-FdU	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: iFluor™ 555-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 (50µL, 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™ FdU Cu-Free Cell Proliferation Fluorescence Imaging Kit uses FOL-FdU, an analog of thymidine. FOL-FdU is incorporated into cellular DNA during DNA synthesis. After fixing cells, the incorporated FOL-FdU is labelled with MTA-iFluor™ 555 through our Buccutite™ labeling technology. The resulted iFluor™ 555-labeled DNA formed in cells is visualized in TRITC Channel. Bucculite™ FdU Cu-Free Cell Proliferation Fluorescence Imaging Kit provides an alternative to anti-BrdU antibody-based assay and EdU click chemistry assay. It is an environment friendly copper-free assay 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 FOL-FdU working solution 100 µL/well for a 96-well plate
3. Incubate at 37 ºC for 3 hours
4. Remove the media and fix cells with 100µL 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™ 555-MTA working solution (100 µL/well) and stain for 30min at room temperature.
7. Remove working solution in each well and wash cells with 1X Washing Buffer three times.
8. Add 100µL 1X washing buffer /well and observe ubder fluorescence microscope with a TRITC filter set.

Important Thaw all the components at room temperature before use.

KEY PARAMETERS

Fluorescence microscope

Excitation	555 nm
Emission	565 nm
Recommended plate	Black wall/clear bottom

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. FOL-FdU stock solution (1000X)
Add 500 µL DMSO (Component E) into FOL-FdU (Component A) to make 1000X stock solution. **Note:** This 1000X concentration was developed with HeLa cells with an optimized FOL-FdU 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.

2. iFluor™ 555-MTA stock solution (400X)
Add 50 µL of DMSO (Component E) to iFluor™ 555-MTA (Component B) to make 400 X iFluor™ 555-MTA stock solution.

PREPARATION OF WORKING SOLUTION

1. FOL-FdU working solution (2X)
Dilute 1000X FOL-FdU stock solution by 500 folds in complete medium to prepare a 2X FOL-FdU working solution.

2. iFluor™ 555-MTA working solution (1X)
Add 2.5 µL 400X iFluor™ 555-MTA stock solution to 1 mL Staining Buffer (Component C) to prepare 1X working solution.

3. Washing Buffer (1X)
Add 1 mL 10X washing buffer (Component D) to 9mL 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 106 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 FOL-FdU

1. Add an equal volume of the 2X FOL-FdU working solution to the volume of media containing cells to be treated to obtain a 1X FOL-FdU 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 FOL-FdU 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.

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

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Stain Cells

1. Add 100 µL/well (96-well plate) or 50 µL/well (384-well plate) of 1X iFluor™ 555-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 well.

3. Wash cells with 1X Washing Buffer three times, and add 100µL washing buffer /well 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 min.

4. Observe the fluorescence signal in cells using fluorescence microscope with a TRITC filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

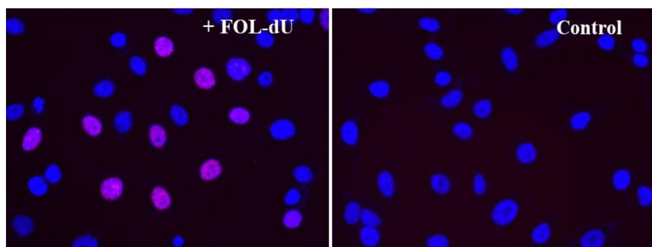

Figure 1. S-phase HeLa cells were detected with Bucculite™ FdU Cu Free Cell Proliferation Fluorescence Imaging Kit (Cat#22315). HeLa cells at 50,000 cells/well/100 µL were seeded overnight in a 96-well black wall/clear bottom plate. Cells were treated with FOL-FdU at 37 ºC for 3 hours, and fixed with Methanol/PBS (90/10). After fixation, cells were stained with iFluor™ 555-MTA for 30min in staining buffer, and then washed three times with 1X washing Buffer. 100µL 5 µg/ml Hoechst 33342 solution in 1X Washing Buffer were added to each well and the fluorescence images were visualized with TRITC filter for S phase cells (Red) and with DAPI filter nuclear for all cells (Blue).

DISCLAIMER

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