EdU DNA Synthesis Monitoring Kit (Microscopy)

(Catalog #NBP2-54871; Store at -20°C)

I. Introduction:

All living cells undergo division cycle, a biological process crucial for proliferation and inheritance. Cell-division cycle is a series of events resulting in two daughter cells containing replicas of DNA from the original DNA molecule. DNA replication occurs in the S phase of the cell cycle and involves *de novo* synthesis of genomic DNA from its precursors. The ability of monitoring detailed characterization of cell cycle and DNA synthesis in proliferating cells is fundamental in basic, and applied immunologic and oncologic studies. Accurate determination of the effect of biologically active reagents on DNA synthesis and cell cycle is of great importance in anti-cancer drug discovery and basic biology. Novus' EdU DNA Synthesis Monitoring Kit utilizes a novel approach that relies on incorporation of 5-EdU (5-ethynyl-2'deoxyuridine) as nucleoside analog to thymidine into newly synthesized DNA directly in the cell culture. Incorporation of EdU into genomic DNA in S-phase is detected based on a click reaction between the alkyne moiety of EdU and fluorescent azide. Compared to historically used BrdU, click reaction is carried in mild conditions and fluorescence microscopy can be used for assessment of proliferating cells in the population. Our kit provides sufficient materials for 50 assays based on the protocol below.

II. Applications:

- Detection of DNA synthesis in proliferating cells and assessment of cell cycle phase
- Screening for genotoxic compounds and effectors of cell division cycle
- Evaluating effects of anti-cancer drugs and genotoxic agents

III. Sample Type:

- Suspension or adherent cell cultures

IV. Kit Contents:

Components	NBP2-54871	Cap Code
Wash Buffer (10X)	25 ml	NM
Fixative Solution	10 ml	WM
Permeabilization Buffer (10X) EdU	25 ml	NM/Blue
DNA Label (1000X) Copper	100 µl	Clear
Reagent (500X)	100 µl	Blue
Fluorescent Azide (100X)	50 µl	Red
Reducing Agent (20X)	1.25 ml	Yellow
Total DNA Stain (1000X)	50 µl	Green

V. User Supplied Reagents and Equipment:

- Tissue culture vessels and appropriate culturing media
- Phosphate Buffered Saline (PBS, pH 7.4)
- Sterile 0.1% Gelatin Solution (optional, only required for suspension cells)
- Fluorescence microscope capable of excitation and emission at 440/490 and 540/580 nm respectively

VI. Storage Conditions and Reagent Preparation:

Upon arrival, store the entire kit at -20°C protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

- **1X Wash Buffer and 1X Permeabilization Buffer:** Dilute the 10X stocks 1:10 in sterile water, mix well. Store at 4 °C, protected from light.
- **Fixative Solution:** Ready to use, after opening store at 4°C, protected from light.
- **Remaining components:** Store at -20°C protected from light. While in use, keep on ice and minimize light exposure.

VII. Assay Protocol:

Notes:

This assay was developed with HeLa and Jurkat cells and can be modified for any suspension or adherent cell line. The protocol below refers to a 12-well tissue culture plate format and the assay volume is **1 ml**. Adjust the volumes accordingly for other plate formats. Growth conditions, cell number per well and other factors might affect cell labeling; therefore conditions should be optimized. We suggest an initial test of several EdU DNA Label concentrations to find best conditions for tested cell type and experimental design. Avoid stressing the cells by washes or temperature changes prior to incubation with EdU DNA Label as it may affect the incorporation rate. Cells should be grown, treated, fixed and stained directly in multi-well plates. Bring all buffers to room temperature prior to the experiment. All steps should be carried out at room temperature unless otherwise specified.

1. Labeling of control and experimental cells:

a. Subculture the cells of interest in appropriate medium to desired density and seed directly into a tissue culture plate or on coverslips. **For suspension cells**; add 1 ml of 0.1% gelatin solution into each well of a 12 well tissue culture plate, tilt the plate to cover the entire well surface and place it in a tissue culture hood for 1 hour. Gently remove the 0.1% gelatin solution and seed your cells. Allow the cells to recover overnight before treatment. Your experiment should always consist of negative and positive controls in parallel to the experimental treatment. Use cells from the same population that was not exposed to the DNA label or treatment as your **negative control** and cells that were incubated with EdU DNA Label only as your **positive control**.

b. Treat the cells with appropriate effectors according to your protocol; do not add treatment to the positive and negative control cells.

c. Add 1 µl of EdU DNA Label to the culture medium of experimental and positive control cells. Incubate for 0.5 - 24 hours in the 37°C incubator, or for the period of time required by your experimental protocol. Note: For longer incubation, decrease the concentration of the DNA label, for shorter incubation times, increase the amount.

d. Terminate the experiment by gentle aspiration of the culture medium. **For suspension cells**: centrifuge the plate at 300 x g (or the lowest centrifuge setting) for 3 minutes to gently deposit the cells onto the surface. Tilt the plate and **gently** remove the media with a pipette tip. It is important to avoid excessive centrifugation speeds, which can damage the cells. *Make note of the place that is used, and perform subsequent aspirations from the same place*.

e. Rinse the cells with 1 ml of Wash Buffer, **For suspension cells:** pellet at 300 x g for 5 minutes and discard the supernatant. Proceed to the Fixation and Permeabilization.

2. Fixation and Permeabilization:

a. Add 0.5 ml of Fixative Solution, mix well and incubate the cells for 15 min at room temperature protected from light.

b. Remove the fixative solution and wash the cells twice in 1 ml of Wash Buffer. **For suspension cells**: pellet the cells to remove the fixative and between the washes at 300 x g for 5 minutes, discard the supernatant.

c. Add 1 ml of Permeabilization Buffer and incubate the cells at room temperature for 20 min. Upon completion, remove the Permeabilization Buffer and wash the cells twice with 1 ml of Wash Buffer. Proceed to reaction and total DNA staining. **For suspension cells**; pellet the cells to remove the Permeabilization Buffer and between the washes at 300 x g for 5 minutes, discard the supernatant.

3. reaction and total DNA staining:

a. Prepare 1X reaction cocktail according to the table below. Volumes should be multiplied by number of samples and reagents added in the exact order. Use the reaction cocktail within 15 minutes of preparation. *Cells should be protected from light during, and following the DNA labeling and staining*.

	Amount per Reaction
PBS	469 µl
Copper Reagent (500X)	1 µl
Fluorescent Azide (100X)	5 µl
Reducing Agent (20X)	25 µl

b. Reaction: Add 500 µl of 1X Reaction cocktail to each sample and incubate the cells for 30 min at room temperature protected from light. Remove the reaction cocktail and wash cells once in 1 ml of Wash Buffer. Remove the wash and suspend the cells in 1 ml of PBS. Proceed to DNA staining. If no staining is desired, proceed to Microscopic analysis. **For suspension cells**: pellet the cells to remove the reaction cocktail and between washes, discard the supernatant. Optional: antibody staining can be performed at this time according to your protocol. *Keep the samples **protected from light** during incubations*.

c. DNA staining: Add 1 µl of Total DNA Stain to each sample and incubate the cells for 20 minutes at room temperature or refrigerate at 4 °C protected from light. Remove the DNA stain solution and wash the cells twice with 1 ml of PBS. **For suspension cells**: pellet the cells to remove the Total DNA Stain and between washes, discard the supernatant.

d. Microscopic analysis: Analyze samples for red fluorescence generated during the click reaction and green fluorescence from the DNA staining respectively. Note: cells are compatible with all methods of slide preparation including wet mount or prepared mounting media.

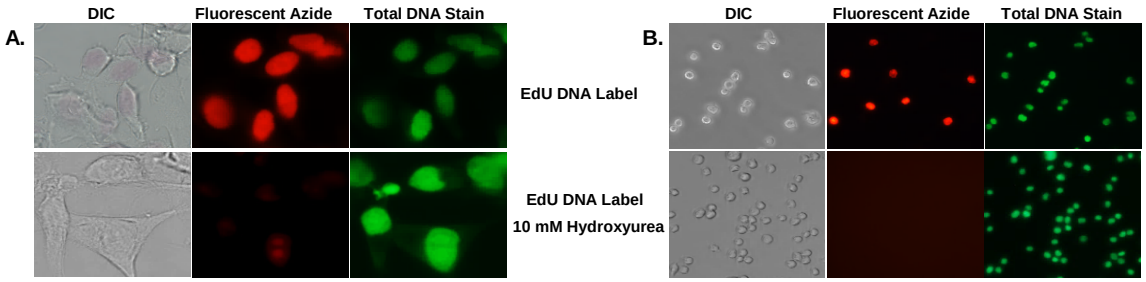

Figure: Detection of EdU DNA Label incorporated into newly synthesized DNA of cultured HeLa (A) and Jurkat (B) cells by fluorescence microscopy. HeLa (10^5 cells/ ml) and Jurkat (10^6 cells/ ml) cells respectively were incubated with 1X EdU DNA Label for 24 hours without (A and B top panels) or in presence (A and B bottom panels) of 10 mM Hydroxyurea to suppress DNA biosynthesis. Fixation and Permeabilization followed by detection with Fluorescent Azide and counterstaining with Total DNA Stain was conducted according to the included protocol. Red fluorescence in top panels of figure A and B reflects number of cells that underwent the S phase and incorporated the EdU Label in their *de novo* synthesized DNA. EdU staining is abolished in cells treated with Hydroxyurea that blocks DNA replication (A and B bottom panels). Nuclear staining in both panels of A and B clearly confirms that red fluorescence is the result of EdU incorporation during DNA synthesis.