

## EZClick™ EdU Cell Proliferation/DNA Synthesis Kit (FACS/Microscopy), Red rev 07/20

(Catalog # K946-100; 100 assays; 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. Biovision's EZClick™ 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 flow cytometry/fluorescence microscopy can be used for assessment of proliferating cells in the population. Our kit provides sufficient materials for 100 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	K946-100	Cap Code	Part Number
EZClick™ Wash Buffer (10X)	25 ml	NM	K946-100-1
Fixative Solution	10 ml	WM	K946-100-2
Permeabilization Buffer (10X)	25 ml	NM/Blue	K946-100-3
EZClick™ EdU DNA Label (1000X)	10 µl	Clear	K946-100-4
Copper Reagent (100X)	100 µl	Blue	K946-100-5
EZClick™ Fluorescent Azide (100X)	100 µl	Red	K946-100-6
Reducing Agent (20X)	500 µl	Yellow	K946-100-7
EZClick™ Total DNA Stain (1000X)	10 µl	Green	K946-100-8

### V. User Supplied Reagents and Equipment:

- Tissue culture vessels and appropriate culturing media; flow cytometry vessels
- Phosphate Buffered Saline (PBS, pH 7.4)
- Sterile 0.1% Gelatin Solution (optional, only required for adhering suspension cells to the surface)
- Flow cytometer equipped with laser capable of excitation at 488 and 530/590 nm emission filters respectively
- 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.

- **10X Wash Buffer and 10X Permeabilization Buffers:** Thaw at 37°C to dissolve completely. Dilute the 10X stocks 1:10 in sterile water, mix well. Store at 4°C.
- **Fixative Solution:** **Divide into aliquots and store at -20°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 (adherent) and Jurkat (suspension) cells and can be modified for any cell line. The protocol below refers to a 96-well tissue culture plate; adjust volumes accordingly for other plate formats. The assay volume is 100 µl. Growth conditions, cell number per well and other factors may affect the incorporation rate of the DNA Label; therefore optimize the assay for your cell type. We suggest an initial test of several EZClick™ 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 EZClick™ EdU DNA Label. All steps should be carried out at room temperature (RT) unless otherwise specified; equilibrated all buffers to RT prior to the experiment.

#### 1. Labeling of control and experimental cells:

- Obtain cell suspension of desired density and seed directly into tissue culture vessels, or on coverslips for high resolution microscopy. **To immobilize suspension cells for microscopy:** add 100 µl of 0.1% gelatin solution directly into the wells, tilt the plate to cover the entire well surface and place it in a tissue culture hood for 1 hour. Gently remove the gelatin solution and seed your cells. Allow the cells to recover overnight before the treatment.
- Next day, remove the media and treat the cells with appropriate effectors according to your protocol; do not add treatment to the positive and negative control cells. **Negative control** (cells not exposed to the DNA Label or treatment), **positive control** (cells incubated with 1X EZClick™ EdU DNA Label only).
- Dilute EZClick™ EdU DNA Label (1000X) to 1X final concentration with culture medium and add into the experimental and positive control cells respectively. Incubate for 0.5 - 24 hours in the 37°C incubator, or for the period of time required by your experimental protocol. For longer incubation, decrease the concentration of the DNA label, or for shorter incubation times, increase the amount.

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d. Terminate the experiment by removal of the culture medium and rinse the cells once with 100  $\mu$ l of PBS, discard the supernatant. **For immobilized suspension cells:** Centrifuge the plate at 500 x g (or the lowest centrifuge setting) for 5 minutes to 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.* Proceed to the Fixation and Permeabilization. *Pellet the suspension cells at 500 x g for 5 min throughout the entire protocol!*

## 2. Fixation and Permeabilization:

a. **For adherent cells:** Add 100  $\mu$ l of Fixative Solution to each well and incubate the cells for 15 min at RT protected from light. Remove the fixative and wash the cells twice with 100  $\mu$ l of 1X Wash Buffer, remove the wash. Add 100  $\mu$ l of 1X Permeabilization Buffer and incubate the cells for 10 min at RT. Remove the Permeabilization Buffer. Proceed to EZClick™ reaction and total DNA staining.

b.

**For suspension cells:** Re-suspend the cells in 100  $\mu$ l of Fixative Solution and incubate for 15 min at RT protected from light. Remove the fixative and wash the cells twice with 0.5 ml of 1X Wash Buffer. Discard the supernatant and re-suspend the cells in 100  $\mu$ l of 1X Permeabilization Buffer. Incubate the cells for 10 min at RT. Remove the Permeabilization Buffer. Proceed to EZClick™ reaction and total DNA staining.

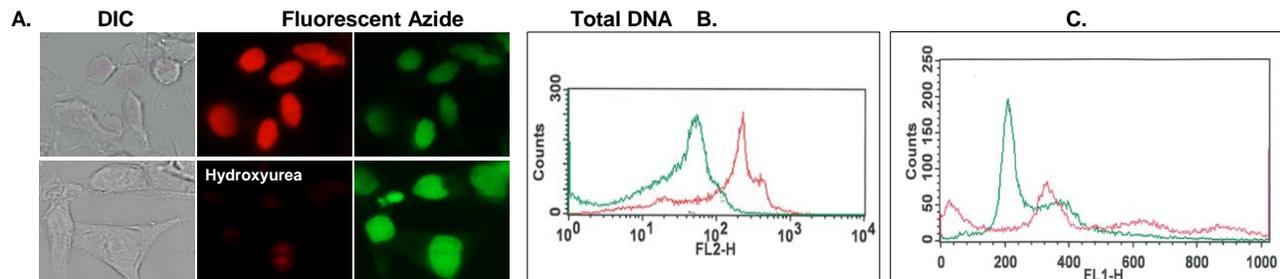
## 3. EZClick™ DNA reaction and total DNA staining:

a. **Reaction cocktail:** Prepare 1X EZClick™ 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 EZClick™ reaction and DNA staining.*

	Amount per Reaction
PBS	93 $\mu$ l
Copper Reagent (100X)	1 $\mu$ l
EZClick™ Fluorescent Azide (100X)	1 $\mu$ l
Reducing Agent (20X)	5 $\mu$ l

b. **EZClick™ Reaction:** For Negative Control Cells: Add 100  $\mu$ l of 1X PBS. For Positive Control Cells and Experimental Cells: add 100  $\mu$ l of 1X EZClick™ 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 three times in 100  $\mu$ l of Wash Buffer. Remove the wash and suspend the cells in 100  $\mu$ l of PBS. Proceed to DNA staining. If no DNA staining is desired, proceed to Microscopic or FACS analysis. **DNA staining:** Prepare 1X dilution of EZClick™ Total DNA Stain and add 100  $\mu$ l per well. Incubate the cells for 20 minutes at room temperature, or refrigerate at 4 °C protected from light. Remove the stain solution; wash the cells once with 100  $\mu$ l of PBS. Note: cells are compatible with all methods of slide preparation including wet mount or prepared mounting media.

4. **Fluorescence Microscope Imaging:** Analyze samples for red fluorescence generated by labeled DNA and green by total DNA respectively. **FACS analysis:** Harvest the cells by preferred method and wash with 0.5 ml of ice-cold PBS. Re-suspend the pellets in 100  $\mu$ l of ice-cold PBS. Transfer the cell suspension into flow cytometry vessels. Analyze samples in FL-2 channel for signal generated by DNA during click reaction. **Note:** Trypsin can be used to collect the adherent cells prior to FACS analysis.



**Figures: Analysis of newly synthesized DNA in proliferating cells by.** (A) HeLa ( $10^5$  cells/ ml) or (B, C) Jurkat ( $10^6$  cells/ ml) cells were incubated with 1X EdU DNA Label for 24 hours in presence and absence of inhibitors of DNA biosynthesis. Red fluorescence reflecting the number of proliferating cells that incorporated the DNA Label in their *de novo* synthesized DNA was analyzed either by fluorescence microscope (A) or FACS (B, C). (A) Treatment with 10 mM Hydroxyurea (bottom panels) suppressed DNA biosynthesis by blocking DNA replication. Total DNA staining in top and bottom panels clearly confirms that red fluorescence is the result of EdU incorporation during cell proliferation. (B) Jurkat cells incubated with vehicle (red) or in presence of 100  $\mu$ g/ml Ganciclovir to suppress DNA biosynthesis (green). Fluorescence measured in FL-2 channel reflects decreased number of proliferating cells treated with Ganciclovir vs. control population. (C) Total DNA content of proliferating cells cultured for 24 h without (green) and with Ganciclovir (red) detected with EZClick™ Total DNA Stain. Fluorescence was measured in FL-1 channel in the linear mode. Ganciclovir arrests proliferating cells in the S phase of the cell cycle. Fixation and Permeabilization followed by detection with EZClick™ Fluorescent Azide™ and counterstaining with EZClick™ Total DNA Stain was conducted according to the kit protocol.

## VIII. RELATED PRODUCTS

EZClick™ Global RNA Synthesis Assay Kit (FACS/Microscopy), Red Fluorescence (K718)  
EZClick™ Global Phospholipid Synthesis Assay Kit (FACS/Microscopy), Red Fluorescence (K717)  
EZCell™ Glutathione Detection Kit (Blue Fluorescence) (K504)

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