ab287859 – EdU DNA Synthesis Monitoring Kit

For the Detection of DNA synthesis in proliferating cells and assessment of cell cycle phase in suspension or adherent cell cultures.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab287859

Storage and Stability

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.

Materials Supplied

Item	Quantity	Storage Condition
EZClick 10X Wash Buffer	25 mL	-20°C
Fixative Solution	10 mL	-20°C
10X Permeabilization Buffer	25 mL	-20°C
EZClick EdU DNA Label (1000X)	10 µL	-20°C
Copper Reagent (100X)	100 µL	-20°C
EZClick Fluorescent Azide (100X)	100 µL	-20°C
20X Reducing Agent	500 µL	-20°C
EZClick Total DNA Stain (1000X)	10 µL	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 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

Reagent Preparation

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.

Assay Procedure

- 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.
- An initial test of several EZClick EdU DNA Label concentrations is suggested 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. Labelling of control and experimental cells
   a. 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.
   b. 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).
   c. 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
   d. Terminate the experiment by removal of the culture medium and rinse the cells once with 100 µl of PBS, discard the supernatant.

2. Fixation and permeabilization
   a. For adherent cells: Add 100 µl 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 µl of 1X Wash Buffer, remove the wash. Add 100 µl 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 µl 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 µl 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. 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 µL
Copper Reagent (100x)	1 µL
EZClick Fluorescenct Azide (100X)	1 µL
Reducing Agent (20X)	5 µL

    b. **For negative Control Cells**: Add 100 µl of 1X PBS. For Positive Control Cells and **Experimental Cells**: add 100 µl 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 µl of Wash Buffer. Remove the wash and suspend the cells in 100 µl of PBS. Proceed to DNA staining. If no DNA staining is desired, proceed to Microscopic or FACS analysis.
    c. **DNA staining**: Prepare 1X dilution of EZClick Total DNA Stain and add 100 µl 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 µl of PBS.
    ΔNote: *cells are compatible with all methods of slide preparation including wet mount or prepared mounting media*

4. Fluorescence Microcope Imaging
    Analyze samples for red fluorescence generated by labeled DNA and green by total DNA respectively.
5. FACS analysis
    Harvest the cells by preferred method and wash with 0.5 ml of ice-cold PBS. Re-suspend the pellets in 100 µl 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.

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
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www.abcam.co.jp/contactus (Japan)