647 Red EdU Click Cell Proliferation Assay (50 &100 Tests; Catalog# TBS2044)

Description

5-ethynyl-2′-deoxyuridine (EdU) is a thymidine analog. It gets incorporated into the newly synthesized DNA of proliferating cells in the place of thymidine. EdU incorporated into the cells can be used directly as an indicator of cell proliferation.

Tribioscience's EdU Click Cell Proliferation Assay Kit detects EdU incorporated with fluorescent labeling via a copper-catalyzed click reaction with a 647-azide red fluorescence. Compared to other cell proliferation assays, this kit detects only the proliferating cells and not the seeded cells. This kit can detect as few as 50-100 proliferating cells and enables rapid, sensitive, and multiplex-compatible detection of proliferating cells via DNA synthesis.

EdU Click Cell Proliferation Assay Kit provides a precise, fast and simple method to quantitate cell proliferation based on the measurement of EdU incorporation during DNA synthesis (S phase) in proliferating cells.

Synonyms: 5-EdU, EdU, 2′-Deoxy-5-ethynyluridine, red fluorescence

Kit Components and Storage for 50 or 100 tests

Part Name	50 tests	100 tests
EdU stock solution (500 x)	12µL	24 µL
647 Red Fluorescence Stock Solution (500x)	11 µL	22 µL
Enhancer (10x)	25 mg	50 mg
FixDenat (1x)	13 mL	26 mL
Wash Reagent (10x)	3 mL	6 mL
Catalyst Solution (25x)	205 µL	410 µL
Click Reaction Buffer	5 mL	10 mL

Storage: -20 °C

Applications

- Detection and quantification of cell proliferation induced by growth factors, cytokines, mitogens, and nutrients.
- Analysis of cytotoxic and cytostatic compounds such as anticancer drugs, toxic agents and other pharmaceuticals.
- Determination of the inhibitory or stimulatory effects of various compounds on cell proliferation.

Sample Types

- Adherent and Suspension cells.

Preparation Instructions

EdU 10 x working solution preparation: Dilute 10 x of EdU working solution by adding 20 µL of EdU stock solution (500x) into 980 µL of medium to for 100 tests or 10 µL of EdU stock solution (500x) into 490 µL of medium for 50 tests. The working solution is used immediately after dilution.

647 Red Fluorescence 100 x working solution preparation: Dilute 500 x of 647 Red Fluorescence Stock to 100x working solution by adding 20 µL 647 Red Fluorescence Stock Solution (500 x) into 80 µL assay buffer for 100 tests or adding 10 µL stock solution (500 x) 40 µL assay buffer to for 50 tests, immediately use it after dilution.

Enhancer working solution: Dissolve Enhancer in 1 mL deionized water for 100 tests or 0.5 mL of deionized water for 50 tests, vortex until completely dissolved, we recommend always using freshly prepared solution.

1x Washing buffer: Dilute 10x Washing Buffer to 1x working solution in 1:10 with deionized water.

EdU Cell Proliferation Assay Protocol

1. **Cell culture:** cells are cultured with the respective test compound in a 96-well plate in a final volume of 100 µL/well at 37°C for required time depending upon the cell type, should ≤70% confluence. Treat cells with desired test compound(s) for 1-24 hrs.
2. **EdU incorporation:** Add 10 µL of 10 x EdU working solution into 90 µL of desired cell, incubate plate at 37°C for 1-2 hours (slow-growing cells may require longer).
3. **Washing:** Remove labeling medium from cells, wash the plate with 150 µL of 1x washing buffer. Then, Aspirate wash buffer from the wells.
4. **Fix and denature:** Add 200 µL of FixDenat Solution into each well. Incubate at room temperature for 30 min.
5. **Washing twice** with 150 µL of 1x washing buffer.
6. **Click Reaction for EdU Detection**
   Prepare assay cocktail for one test: by adding 1 µL of 647 Red Fluorescence azide (100x) working solution, 4 µL of Catalyst Solution (25x), 10 µL of Enhancer working solution (10x) and 85 µl of Click Reaction Buffer, add 100µL per coverslip for imaging.
7. **Incubate 30 minutes** at room temperature, protected from light.
8. **Optional Co-staining DNA dyes** (DAPI, PI, 7-ADD) for cell circle analysis.
9. **Mounting the coverslip.**
10. **Take the images in the microscope and analysis.**

Note: 1. The click reaction cocktail contains copper, which is known to quench the fluorescence of R-PE. Antibody conjugates containing PE or R-PE should be stained after the click reaction. 2. The copper present in the click reaction cocktail may affect binding of anti-GFP antibodies to their epitopes. We recommend staining with anti-GFP antibodies before the click reaction. 3. Organic dyes (e.g. Alexa Fluor® dyes), PerCP-Cy™5.5, APC, Brilliant™ Violet, and Brilliant™ Ultraviolet dyes have been found to be compatible with the click reaction. 4. Large shifts in autofluorescence can occur from exposure to the click component cocktail. We recommend that all controls, including unstained and single stained controls, also be treated with the click component cocktail with or without the dye azide as appropriate so that control and test samples exhibit the same auto fluorescent properties.

For suspension cells:

A. For suspension cells, centrifuge plate at 300 x g for 10 min. and remove medium carefully before adding FixDenat.

B. Incubation time after addition of Substrate must be optimized to avoid over development of color.

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Related Product:

Resazurin Cell Viability Kit (TBS2001)
LDH Cytotoxicity Assay (TBS2002)
ATP Colorimetric/Fluorometric Assay Kit (TBS2010)
ADP Colorimetric/Fluorometric Assay Kit (TBS2020)
Caspase-3 Colorimetric Assay kit (TBS2030)
Alkaline Phosphatase Staining kit I-Red (TBS2080)
Cytochrome C Oxidate Assay (TBS2115)
Cytochrome c Reductase Activity Assay (TBS2116)
Non-esterified Fatty Acid Assy (TBS2203)
Glycerol Colorimetric / Fluorometric Assy (TBS2204)
WST-8 or Cell Count Kit-8 (CCK-8) (TBS2022)
Brdu Cell proliferation assay (TBS2082)

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