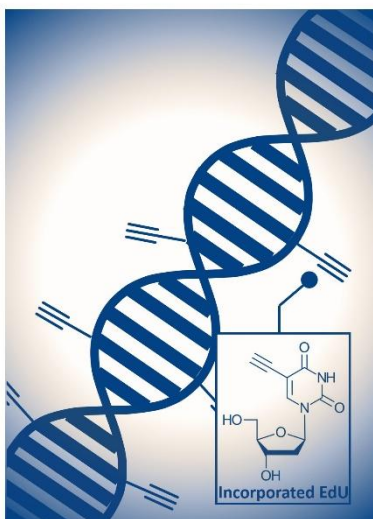
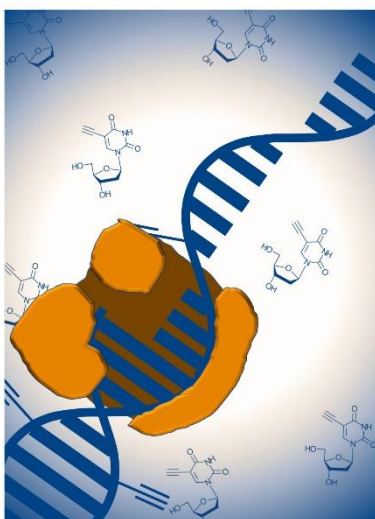




User Manual

ClickTech EdU Cell Proliferation Kit for Imaging



Ordering information
(for detailed kit content see **Table 1**)

ClickTech EdU Imaging Kits:

| Product number | EdU | Used fluorescent dye |
|-----------------|------|--|
| BCK-EdU488IM100 | 5 mg | 6-FAM Azide (FITC alternative) |
| BCK-EdU555IM100 | 5 mg | 5-TAMRA-PEG3-Azide (Cy3 alternative) |
| BCK-EdU594IM100 | 5 mg | 5/6-Sulforhodamine 101-PEG3-Azide (Texas Red alternative) |
| BCK-EdU647IM100 | 5 mg | Eterneon-Red 645 Azide (Cy5 Azide alternative) |

For References, FAQs and ordering please see online or contact us:

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ClickTech EdU Cell Proliferation Kit for Imaging

The *ClickTech EdU Imaging Kit* contains chemicals to perform 100 reactions (500 μ L each).

Introduction and product description:

The detection of cell proliferation is of utmost importance for assessing cell health, determining genotoxicity or evaluating anticancer drugs. This is normally performed by adding nucleoside analogs like [3 H]thymidine or 5-bromo-2'-deoxyuridine (BrdU) to cells during replication, and their incorporation into DNA is detected or visualized by autoradiography or with an anti-BrdU-antibody respectively. Both methods exhibit several limitations. Working with [3 H]thymidine is troublesome because of its radioactivity. Autoradiography is slow and thus not suitable for rapid high-throughput studies. The major disadvantage of BrdU staining is that the double-stranded DNA blocks the access of the anti-BrdU antibody to BrdU units. Therefore samples have to be subjected to harsh denaturing conditions resulting in degradation of the structure of the specimen.

The baseclick *ClickTech EdU-Click Assays* overcome these limitations, providing a superior alternative to BrdU and [3 H]thymidine assays for measuring cell proliferation. EdU (5-ethynyl-2'-deoxyuridine) is a nucleoside analog to thymidine and is incorporated into DNA during active DNA synthesis. In contrast to BrdU assays, the *EdU-Click Assays* are not antibody based and therefore do not require DNA denaturation for detection of the incorporated nucleoside. Instead, the *EdU-Click Assays* utilize click chemistry for detection in a variety of dye fluorescent readouts. Furthermore, the streamlined detection protocol reduces both the total number of steps and significantly decreases the total amount of time. The simple click chemistry detection procedure is complete within 30 minutes and is compatible with multiplexing for content and context-rich results.

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Literature Citation: When describing a procedure for publication using this product, please refer to it as the *ClickTech EdU-Imaging kit*.

1. Materials provided with the Kit and storage conditions

Table 1: Contents of the kit and storage conditions

| Component code | Amount | Component | Component long term storage | Kit storage |
|----------------------------|---------------|---|-----------------------------|--|
| Component E yellow | 5 mg | 5-Ethynyl-deoxyuridine (5-EdU) | – 20 °C | 2 - 8 °C Dark Do not freeze Dry |
| Component D red | 130 µL | <ul style="list-style-type: none"> 6-FAM Azide (BCK-EdU488) 5-TAMRA-PEG3-Azide (BCK-EdU555) 5/6-Sulforhodamine101-PEG3-Azide (BCK-EdU594) Eterneon-Red 645 Azide (BCK-EdU647) | – 20 °C dark | |
| Component S | 5 mL | DMSO | RT | |
| Component RB orange | 4 x 2 mL | Reaction buffer (10x) | 2 - 8 °C | |
| Component C green | 2 x 2 mL | Reactor System | RT | |
| Component B blue* | 4 x 200 mg | Buffer additive* | – 20 °C* | |
| Compound K white | 100 µL | 300 µM DAPI solution | – 20 °C | |

This kit is stable up to 1 year after receipt, when stored as directed.

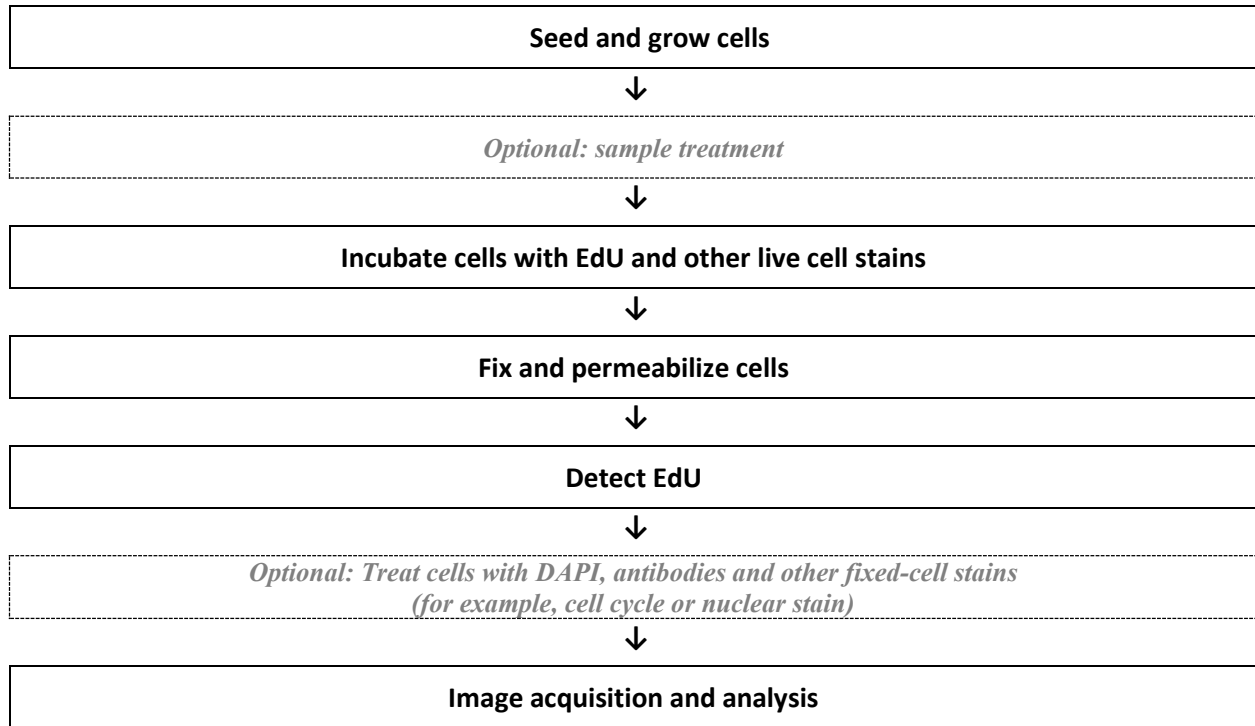
* When dissolved the component has to be kept at – 20 °C for long-term storage.

2. Required Material and Equipment not included in this Kit

- Cells adherently grown on a coverslip
- Reaction tubes (size depends on the volume of reaction cocktail needed)
- Phosphate-buffered saline (PBS, pH 7.2 - 7.6)
- Appropriate cell culture medium
- Fixation solution (3.7% formaldehyde in PBS)
- Permeabilization solution (for example, 0.5% Triton® X-100 in PBS)
- 3% BSA (bovine serum albumine) in PBS (3% BSA in PBS), pH 7.4
- Deionized water
- 18 x 18-mm coverslips
- *Optional:* 6-well microplate

3. Workflow

Workflow scheme for the EdU Imaging assays



4. Preparation of the stock solutions

- 4.1. Allow all vials to warm to room temperature before opening.
- 4.2. Prepare 10 mM stock solution of EdU (Component E): Add 2 mL of DMSO (Component S) and mix until the compound is dissolved completely. After use, store any remaining solution at – 20 °C. When stored as directed, this stock solution is stable for up to one year.
- 4.3. Prepare a 10x stock solution of the buffer additive (Component B): Add 2 mL of deionized water to each of the blue vials and mix until the compound is dissolved completely. After use, store any remaining solution at – 20 °C. When stored as directed, this stock solution is stable for up to 6 months. If the solution starts to develop a brown colour, it has degraded and should be discarded. We recommend to prepare aliquots to avoid repeated thaw and freeze cycles!

5. Labeling of cells with EdU

This protocol can be adapted for any adherent cell type. An EdU concentration of 10 μM is a good starting concentration. Cell type variations, cell density, growth medium and other factors may influence the labelling.

- 5.1. Seed cells on cover slips and grow them until the desired density (typical \approx 80% confluence).
- 5.2. Prepare a 2x working solution of EdU in fresh medium from the 10 mM EdU stock solution (Component E). If you start with a 10 μM final concentration of EdU, prepare a 2x working solution of 20 μM .
- 5.3. Pre-warm the 2x EdU solution and mix it with the same volume of adapted medium from the coverslips to obtain a 1x EdU solution. We do not recommend to replace all of the media with fresh media, because this could affect the rate of cell proliferation.
- 5.4. Aspirate the rest of medium from the coverslips and add the 1x EdU solution.
- 5.5. Incubate the cells for the desired pulse length under conditions optimal for the cell type.
- 5.6. Proceed immediately to the cell fixation and permeabilization step 6.

6. Cell fixation and permeabilization

This protocol was developed with a fixation step using 3.7% formaldehyde in PBS, followed by a 0.5% Triton[®] X-100 permeabilization step, but it is also amenable to other cell fixation/permeabilization reagents. For a better handling and processing, we recommend to transfer the coverslips into a 6-well plate, so that each well contains a single coverslip.

- 6.1. After incubation, remove the media and add 1 mL 3.7% formaldehyde in PBS (fixation solution) to each well containing the coverslips. Incubate for 15 minutes at room temperature.
- 6.2. Remove the fixation solution and wash the cells in each well twice with 1 mL of 3% BSA in PBS.
- 6.3. Remove the wash solution and add 1 mL of 0.5% Triton[®] X-100 in PBS (permeabilization solution) to each well. Incubate for 20 minutes at room temperature.

7. EdU detection

In this protocol, 500 μL of the reaction cocktail per coverslip are used. Also smaller volumes can be used, as long as the reaction components are applied in the same ratios.

- 7.1.** Prepare the reaction cocktail in the same order as described in the following table. If the ingredients are not added in the order listed, the reaction will not proceed optimally or might even fail. Important: Once the reaction cocktail is prepared, use it immediately, at least within the next 15 minutes!

Reaction cocktail per coverslip (500 μL):

Table 2: Click assay cocktails

| Material | Component code | Number of assays | | | |
|--|----------------|-------------------------------------|-------------------|--------------------|--------------------|
| | | 1 | 2 | 5 | 10 |
| Deionized water | Not provided! | 379 μL | 758 μL | 1895 μL | 3790 μL |
| Reaction buffer (10x) | RB | 50 μL | 100 μL | 250 μL | 500 μL |
| Reactor System | C | 20 μL | 40 μL | 100 μL | 200 μL |
| Dye Azide (10 mM) | D | 1 μL | 2 μL | 5 μL | 10 μL |
| Buffer additive (10x) (prepared in 4.3) | B | 50 μL | 100 μL | 250 μL | 500 μL |
| Total Volume | - | 500 μL | 1 mL | 2.5 mL | 5 mL |

- 7.2.** Remove the permeabilization solution, then wash the cells in each well twice with 1 mL of 3% BSA in PBS. Remove the wash solution.
- 7.3.** Add 500 μL of reaction cocktail to each well containing a coverslip. Rock the plate gently to distribute the reaction cocktail evenly over the coverslip.
- 7.4.** Incubate the plate for 30 minutes at room temperature. Protect from light!
- 7.5.** Remove the reaction cocktail, then wash the cells in each well three times with 1 mL of 3% BSA in PBS. Remove the wash solution.

8. Optional: Cell nucleus staining

Proceed with nuclear staining with the included DAPI (Component K) or antibody labeling. Important: Keep the samples protected from light during incubations. If no additional staining is desired, proceed with imaging and analysis.

- 8.1. Wash the cells with PBS and remove the wash solution.
- 8.2. Create a 300 nM solution by diluting the amount needed of the 300 μ M DAPI Stock solution by diluting 1 to 1000 with PBS (e.g. 1 μ L DAPI and 999 μ L of PBS). Prepare enough DAPI solutions to cover your cells. Usually 0.5-1 ml of 300 nM DAPI solution per well is needed, the amount should cover your cells with liquid. Incubate 5 min in the dark. Diluted solutions can be stored at -20 °C in the dark. We recommend creating at least 1 mL of solution.
- 8.3. Remove the DAPI solution and then wash the cells three times with PBS and continue with point 9.

9. Imaging and analysis

EdU Click cells are compatible with all methods of slide preparation, including wet mount or prepared mounting media.

The Excitation and emission maxima of the available dyes are listed in the following **Table 3**.

Table 3: Emission and excitation maxima of the available dyes

| Product number | Dye | Excitation (nm) | Emission (nm) | Filter |
|-----------------|---|-----------------|---------------|--------|
| BCK-EdU488IM100 | 6-FAM Azide | 496 | 516 | green |
| BCK-EdU555IM100 | 5-TAMRA-PEG3-Azide | 546 | 579 | yellow |
| BCK-EdU594IM100 | 5/6-Sulforhodamine 101-PEG3-Azide | 584 | 603 | orange |
| BCK-EdU647IM100 | Eterneon-Red 645 Azide (Cyanine 5 Azide analogue) | 643 | 662 | red |