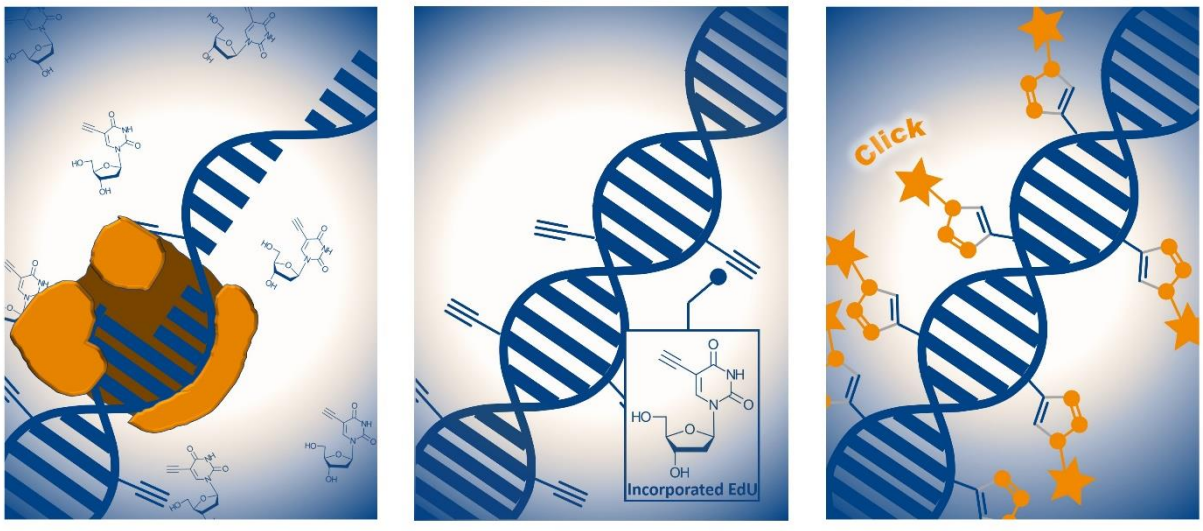
baseclick
DNA & RNA EXPERTS

User Manual

ClickTech EdU Cell Proliferation Kit for Imaging



Version 5.0

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 µL 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	• 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*	

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

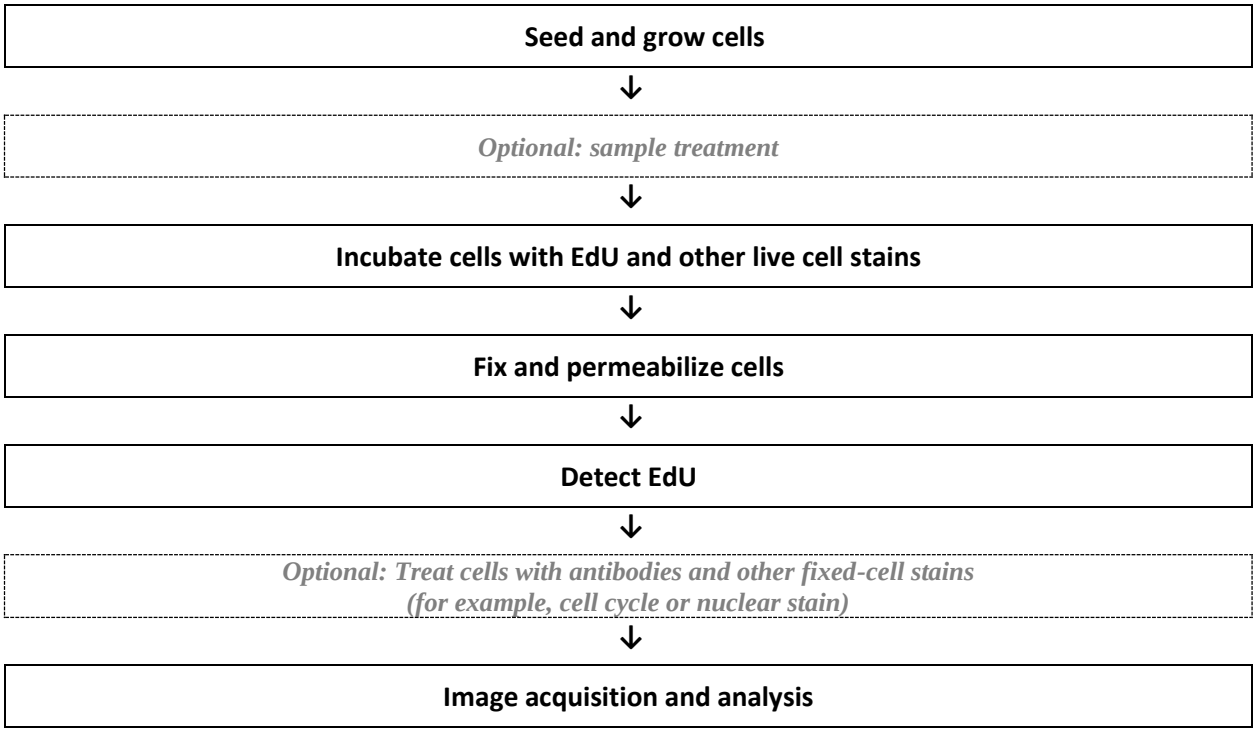
* When dissolved the component has to be kept at – 20 °C for long-term storage.

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 ≈ 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.

Optional: Proceed with nuclear staining (DAPI or Hoechst 33342) or antibody labeling. Important: Keep the samples protected from light during incubations. If no additional staining is desired, proceed with imaging and analysis.

8. 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**