User Manual

ClickTech Sensitive EdU Cell Proliferation Kit for Imaging

Version 3.0

Ordering information

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

ClickTech Sensitive EdU Imaging Kits:

Product number	EdU	Used fluorescent dye
BCK-EdUPro488IM100	5 mg	Eterneon² GREEN Azide (Enhancer system – incl. FITC alternative)
BCK-EdUPro647IM100	5 mg	Eterneon² RED Azide (Enhancer system – incl. Cy5 alternative)

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

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

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ClickTech Sensitive EdU Cell Proliferation Imaging Kit

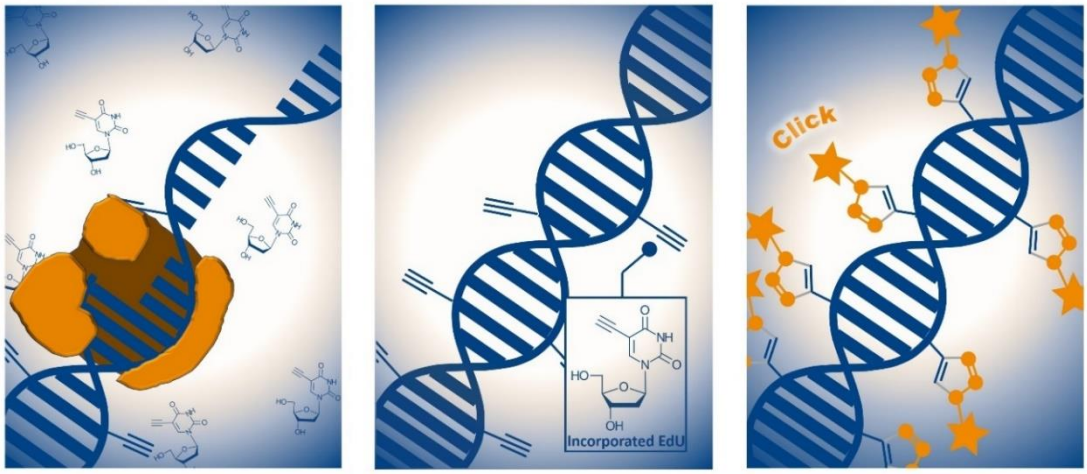
Introduction and product description:

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 analogues 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.

For research use only.

Literature Citation: When describing a procedure for publication using this product, please refer to it as the *ClickTech Sensitive EdU Imaging Kit* from baseclick GmbH.

How the **enhanced ClickTech Sensitive EdU cell proliferation** assay works



The baseclick ***Sensitive EdU*** overcome these limitations, providing a superior alternative to BrdU and [^{3}H]thymidine assays for measuring cell proliferation.

Just as in the traditional EdU proliferation kits from baseclick, also here EdU (5-ethynyl-2'-deoxyuridine) (a nucleoside analog to thymidine) is incorporated into DNA during active DNA synthesis. In contrast to BrdU assays, the ***Sensitive EdU*** are not antibody based and therefore do not require DNA denaturation for detection of the incorporated nucleoside. Instead, the ***Sensitive EdU*** 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.

This **enhanced *Sensitive EdU*** assay is combining all the above advantages of the standard EdU assay, with higher sensitivity and brightness (signal to background ratio) due to the novel enhancer system in the kit, without altering the easiness of the protocol.

EdU ***Sensitive EdU***

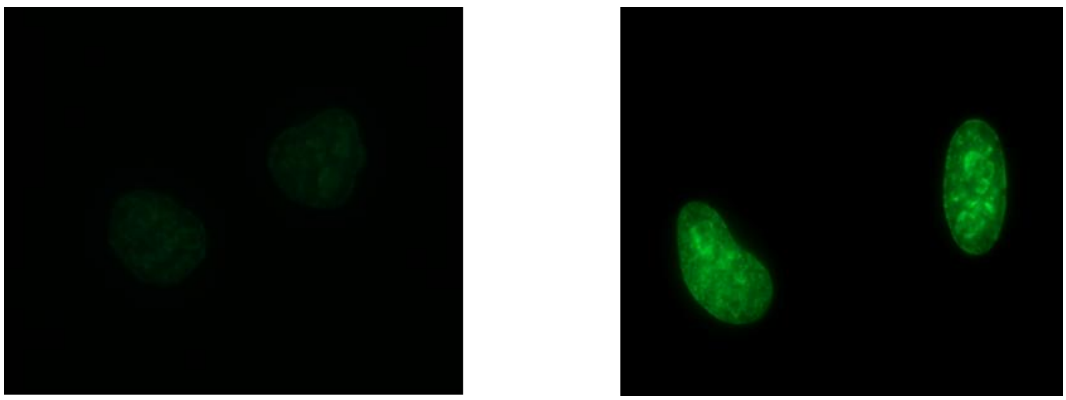


Figure 1: Incubation of HeLa cells for 1 hour with EdU. The subsequent detection of cell proliferation was done using the standard EdU labeling kit and the enhanced BCK-EdUPro488IM100; exposure time = 15 ms.

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 short term storage*
Component E yellow	5 mg	5-Ethynyl-deoxyuridine (5-EdU)	– 20 °C	2 - 8 °C Dark Do not freeze Dry
Component D red	2 x 60 µL	• Eterneon² GREEN Azide (BCK-EdUPro488IM100) • Eterneon² RED Azide (BCK-EdUPro647IM100)	– 20 °C dark	
Component S	5 mL	DMSO	RT	
Component C green	2 x 2 mL	Reactor System	2 - 8 °C	
Component P	6 mL	Saponin-based reagent (10x solution)	2 - 8 °C	
Component F	55 mL	Fixative solution (4% Paraformaldehyde)	2 - 8 °C	
Component B blue	4 x 200 mg	Buffer additive	– 20 °C**	
Component RB orange	4 x 2 mL	Reaction buffer (10x)	2 - 8 °C	

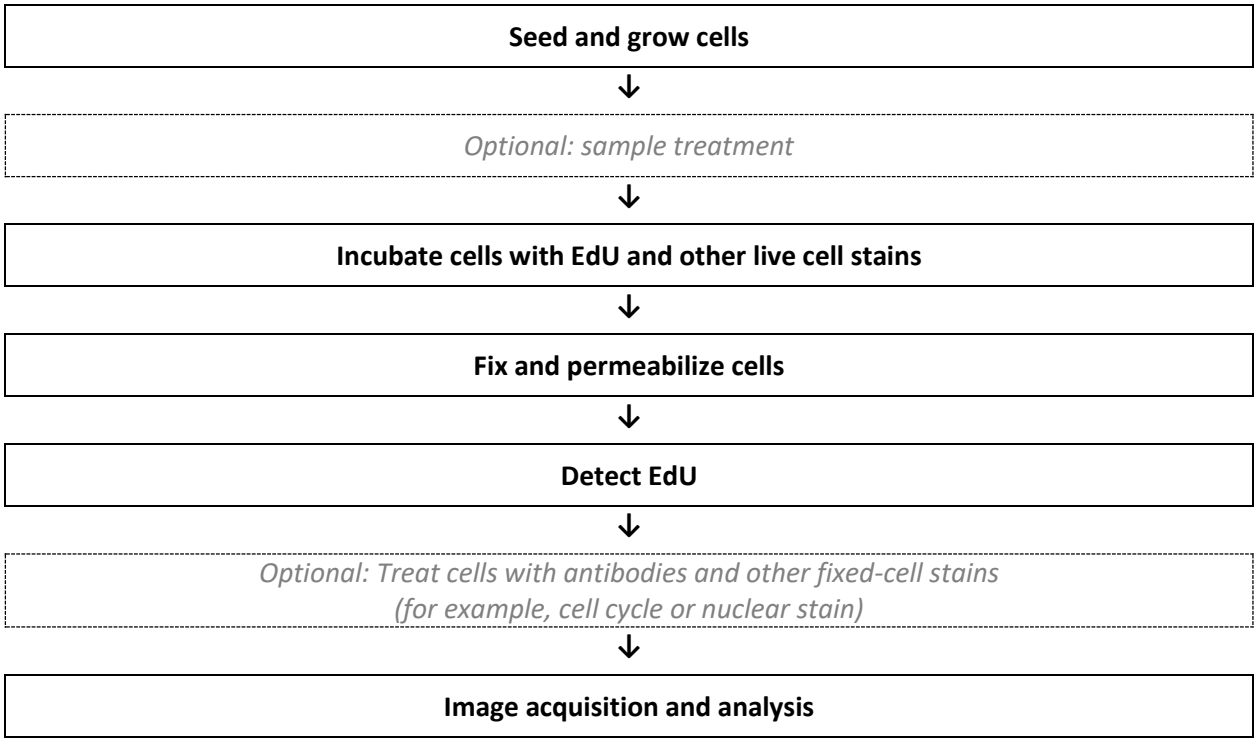
* This kit is stable up to 1 year after receipt, when stored as directed.

** When dissolved the component has to be kept at – 20 °C for long-term storage. Prepare aliquots to avoid too many freeze and thaw cycles; if the solution starts to develop a brown colour, it has degraded and should be discarded.

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
- 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



4. Preparation of the stock solutions

4.1. Allow all vials to warm to room temperature before opening.

4.2. **Prepare a 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 **Component B** 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!

4.4. **Dilution of the Saponin based reagent (10X solution) (Component P):**

To prepare 60 mL of saponine-based solution add 54 mL of PBS 1x to 6 mL of saponin 10x solution. A smaller amount can be prepared by diluting a volume of **Component P** with PBS 1x.

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. If currently using a BrdU based assay for cell proliferation, a similar concentration to BrdU is a good starting concentration for EdU. Cell type variations, cell density, growth medium and other factors may influence the labelling.

For the time of incubation, please consider the division rate of your cells. The cells have to have the chance to divide during the time of EdU incubation.

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 (e.g. for a final concentration of 10 µM replace half volume of the media with 2x 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. Incubate the cells for the desired pulse length under conditions optimal for the cell type. If you previously worked e.g. with BrdU staining, we recommend starting with the same incubation time.

5.5. Proceed immediately to the cell fixation and permeabilization step **6**.

6. Cell fixation and permeabilization

This protocol was developed with a fixation step using 4% formaldehyde in PBS, followed by a Saponin 1x based 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 500 µL fixation solution (4% formaldehyde in PBS **Component F**) 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 500 µL of permeabilization solution (Saponin 1x **Component P**) 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	D	1 µL	2 µL	5 µL	10 µL
Buffer additive (10x) (from *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 500 µL 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

ClickTech Sensitiv 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-EdUPro488IM100	Eterneon^2 GREEN Azide (Enhancer system – incl. FITC alternative)	496	516	green
BCK-EdUPro647IM100	Eterneon^2 RED Azide (Enhancer system – incl. FITC alternative)	643	662	red