# User Manual

# EdU DetectPro Cell Proliferation HTS Kit

Version 3.0

Ordering information

(for detailed kit content see Table 2)

**EdU DetectPro HTS Kits ready for 2 x 96 well plate assays:**

| Product number | EdU | Used fluorescent dye |
|---|---|---|
| **BCK-EdUPro-HTS488-200** | 2 mL | Eterneon$^2$ **GREEN Azide** **(**Enhancer system – incl. **FITC alternative)** |
| **BCK-EdUPro-HTS555-200** | 2 mL | Eterneon$^2$ **YELLOW Azide** **(**Enhancer system – incl. **Cy3 Azide alternative)** |

The Kit contains sufficient material for 2 x 96 well plate assays

**EdU DetectPro HTS Kits ready for 4 x 96 well plate assays:**

| Product number | EdU | Used fluorescent dye |
|---|---|---|
| **BCK-EdUPro-HTS488-400** | 2 x 2 mL | Eterneon$^2$ **GREEN Azide** **(**Enhancer system – incl. **FITC alternative)** |
| **BCK-EdUPro-HTS555-400** | 2 x 2 mL | Eterneon$^2$ **YELLOW Azide** **(**Enhancer system – incl. **Cy3 Azide alternative)** |

The Kit contains sufficient material for 4 x 96 well plate assays

For references and FAQs see online

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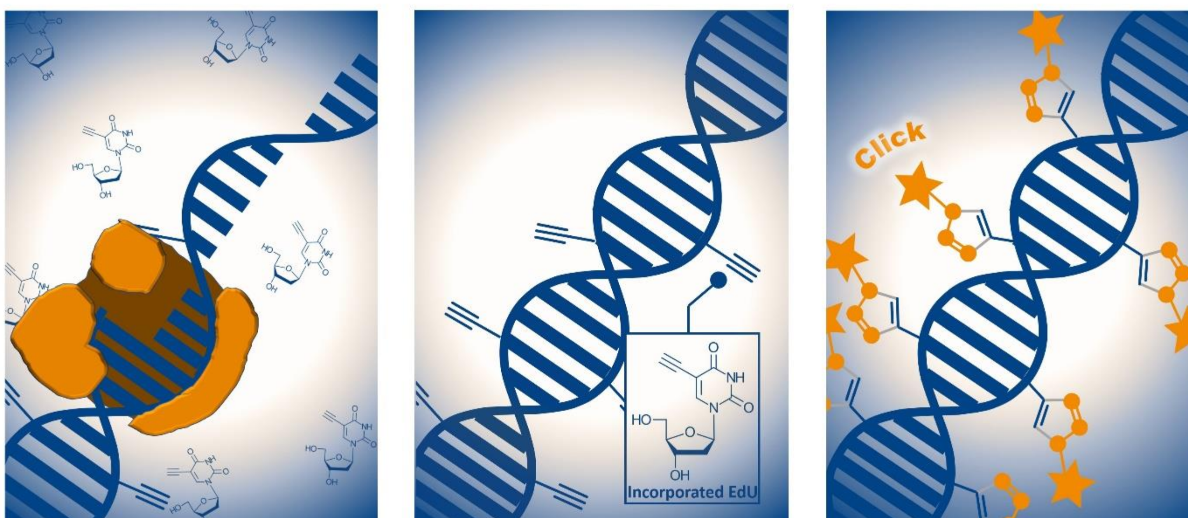
**EdU DetectPro HTS Kit**

**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.

How the **enhanced *EdU DetectPro* cell proliferation** assay works




The baseclick ***EdU DetectPro*** 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 ***EdU DetectPro*** are not antibody based and therefore do not require DNA denaturation for detection of the incorporated nucleoside. Instead, the ***EdU DetectPro*** 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.

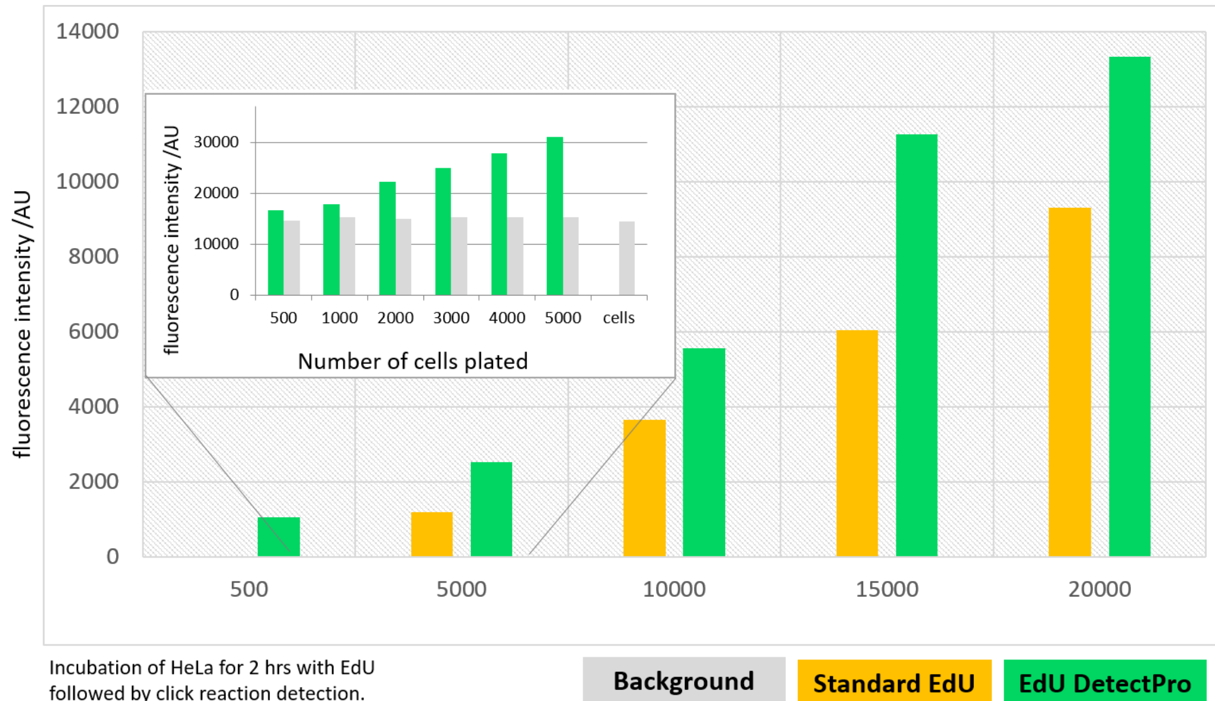



**Figure 1:** Comparison of the fluorescence intensities between the standard EdU Kit and the EdU DetectPro Kit.

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Please read the material safety data sheets (MSDS) provided for each product/component.

**Literature Citation:** When describing a procedure for publication using this product, please refer to it as *baseclick EdU DetectPro HTS Kit*.

The baseclick *EdU DetectPro HTS Kit* can be used with antibodies against surface and intracellular markers. To ensure the compatibility of your reagent or antibody, please refer to **Table 1**.

**Table 1:** EdU detection dye compatibility

| Fluorescent molecule | Compatibility* |
|---|---|
| Organic dyes such as Fluorescein and Alexa dyes | Compatible |
| PerCP, Allophycocyanin (APC) and APC-based tandems | Compatible |
| R-phycoerythrin (R-PE) and R-PE based tandems | Use R-PE and R-PE based tandems **after** the EdU detection reaction |
| Quantum Dots | Use Quantum Dots **after** the EdU detection reaction |
| Fluorescent proteins (e.g. GFP) | Use anti-GFP antibodies** **before** the EdU detection reaction or use organic dye-based reagents for protein expression detection |

* Compatibility indicates which involved components are unstable in the presence of copper catalyst for the EdU detection reaction (either the fluorescent dye itself or the detection method).

** The resulting fluorescence intensity depends strongly on the antibody manufacturer and target. Internal tests have shown a generally good fluorescence amount for rabbit and chicken anti-GFP and a very low fluorescence amount for mouse monoclonal antibodies. This can be understood as a general guideline but results may still greatly vary depending on the individual chosen antibody.

## 1. Materials provided with the Kit and storage conditions

**Table 2:** Contents of the kit and storage conditions

| Vial-label | Amount for 2 assays/well plates | Amount for 4 assays/well plates | Component | Component long term storage | Kit short term storage* |
|---|---|---|---|---|---|
| **Component A**<br>**yellow** | 2 mL | 2 x 2 mL | 5-Ethynyl-deoxyuridine (5-EdU) | -20 °C | 2 – 8 °C<br>Dark<br>Do not freeze<br>Dry |
| **Component B**<br>**red** | 1 x 60 µL | 1 x 120 µL | Eterneon[2] GREEN Azide (BCK-EdUPro-HTS488)<br>Eterneon[2] YELLOW Azide (BCK-EdUPro-HTS555) | -20 °C<br>dark | |
| **Component C**<br>**orange** | 20 mL | 40 mL | Reaction buffer | 2 – 8 °C | |
| **Component D**<br>**green** | 2 mL | 2 mL | Reactor system | 2 – 8 °C | |
| **Component E**<br>**blue** | 200 mg | 400 mg | Buffer additive | 2 – 8 °C/<br>- 20 °C** | |
| **Component F*** ** | 6 mL | 2 x 6 mL | Rinse buffer (10x) | RT | |

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

** When dissolved the component E 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.

***** Cautions:**

*The rinse buffer* (**Component F**): contains hazardous components. Use with appropriate precautions. Keep away from acids to avoid dangerous gases.

Handle reagents containing the rinse buffer using equipment and practices appropriate for the hazards posed by such materials. Use gloves. Dispose of the reagents in compliance with all related local arrangements. For the correct handling, we refer you to the MSDS that can be downloaded from our webpage www.baseclick.eu

This solution is stored at RT and will crystallize at lower temperatures. If crystallized, the solution has to be brought to RT, mixed thoroughly and can then, once homogenously dissolved, be used without further considerations. The activity of this compound is not affected hereby.

**MSDS:** the appropriate MSDS can be downloaded from our website www.baseclick.eu .

## 2. Required Material and Equipment ***not included*** in this kit

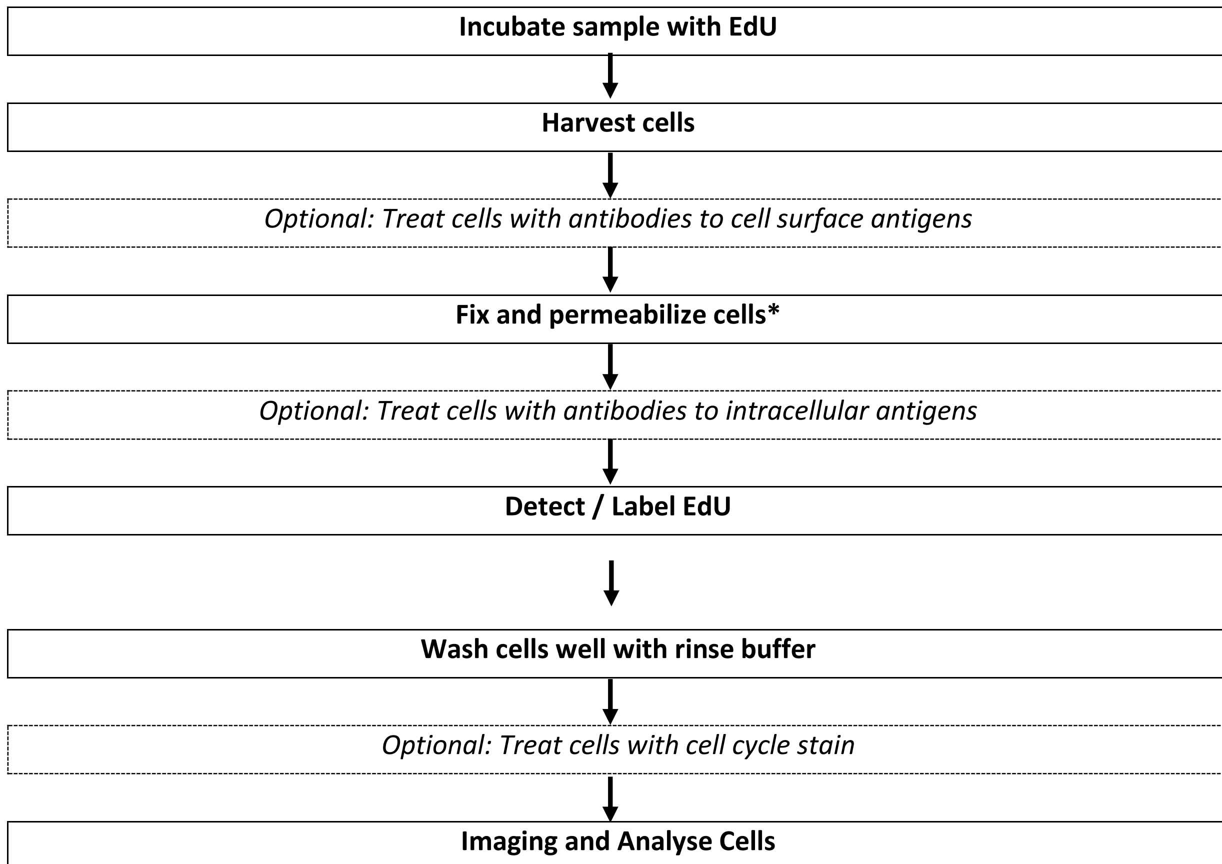
- Adherent cells
- Reaction tubes (size depends on the volume of reaction cocktail needed)
- Buffered saline solution, such as PBS, D-PBS or TBS
- Fixative solution (4 % Paraformaldehyde in PBS)
- Permeabilization solution optimised for your cell line (for example, 0.5 % Triton® X-100 in PBS, or a 0.5 % saponin-based solution)
- Appropriate cell culture medium
- 1 % BSA (bovine serum albumin) in PBS, pH 7.1 – 7.4
- Deionized water or 18 MΩ purified water

## 3. Workflow

The following protocol was developed using a final EdU concentration of 10 µM and can be adapted for any cell type. There are many factors, which can influence the labeling such as the growth medium, the density and the type of cells. To determine the optimal concentration for your experiment, a range of EdU concentrations should be tested for your cell type and experimental conditions.

Principally, a similar concentration to BrdU can be used for EdU as a starting point. Heparin can be used as anticoagulant for collection, if a whole blood sample is used.

**Workflow scheme for the *EdU HTS Assay***




* At this point, the sample can be stored safely.

## 4. Preparation of the stock solutions

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

**4.1.1** For the preparation of a 20 µM stock solution of EdU (2x EdU), add the appropriate amount of aqueous solution (1x PBS) to EdU (**component A**) according to **table 3** and mix until the compound is completely dissolved. After use, store any remaining solution at -20 °C. When stored as directed, this stock solution is stable for up to one year.

**Table 3:** Amounts of aqueous solution needed to dissolve EdU to a final concentration of 20 µM

| EdU DetectPro HTS Kit | 20X EdU solution | Dilution volume of 1x PBS |
|---|---|---|
| **1 well plate** | 1 mL | 9 mL |
| **2 x 96 well plates** | 2 mL | 18 mL |
| **4 x 96 well plates** | 4 mL | 36 mL |

**4.1.2** For the preparation of a stock solution of the buffer additive, add the appropriate amount of deionized water (see table 4) to the **component E** 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. We recommend preparing aliquots to avoid repeated freeze and thaw cycles!

**Table 4:** Amounts of aqueous solution needed to dissolve the buffer additive to the final work solution

| EdU DetectPro HTS Kit | Buffer additive (solid) | Dilution volume of deionized water |
|---|---|---|
| **1 well plate** | 100 mg | 1 mL |
| **2 x 96 well plates** | 200 mg | 2.5 mL |
| **4 x 96 well plates** | 400 mg | 5 mL |

**5. Labeling of cells with EdU**

**5.1** Suspend the cells in an appropriate tissue culture medium to obtain optimal cell growth conditions. Please note that the growth of the cells during incubation decelerates, if the temperature changes or the cells are washed prior to incubation with EdU.

**5.2** For the desired final concentration, add the appropriate amount of EdU to the culture medium and mix well. We recommend using a concentration of 10 µM for 1-4 hours as a starting point. Use higher EdU concentrations for a shorter incubation time. A longer incubation time requires lower EdU concentrations.

**5.3** The incubation of the cells with EdU should be performed under the optimal conditions for your cell type, the number of cells plated and for the desired length of time. Various DNA synthesis and proliferation parameters can be evaluated by altering the EdU incubation time or by subjecting the cells to pulse labeling with EdU. Effective time intervals for pulse labeling and the length of each pulse depend on the cell growth rate and the number of cells used.

**5.4** If performing antibody surface labeling, proceed immediately to step **6**, otherwise continue to step **7**.

***6. Staining cell-surface antigens with antibodies (optional)***

**6.1** Wash cells in each well with 100 µL of 1 % BSA in PBS.

**6.2** Remove the wash solution and add again 100 µL of 1 % BSA in PBS to the cells.

**6.3** Add surface antibodies and mix well but gently.

**Note:** PE, PE-tandem or Quantum Dot antibody conjugates should not be used before performing the click reaction (step **8**).

**6.5** Incubate the cells for the recommended length of time and temperature. Protect from light!

**6.6** Proceed to step **7**.

### 7. Cell fixation and permeabilization

This protocol was developed with a fixation step using 4 % Paraformaldehyde in PBS, followed by permeabilization step. A saponin-based permeabilization solution can be used with cell samples containing red blood cells or whole blood as well as with cell probes containing different cell types. The morphological light scatter characteristics of leukocytes are maintained by a saponin-based solution while red blood cells are lysed.

**7.1** Remove the incubation media and wash the cells, each well with **100 µL of 1 % BSA in PBS.** Afterwards remove the wash solution.

**7.2** Add **100 µL of the fixative solution** to the cells in each well. Incubate for **15 minutes at room temperature**. Protect from light.

**7.3** Remove the fixation solution and wash the cells in each well twice with **200 µL of 1 % BSA in PBS**. If red blood cells or haemoglobin are present in the sample, repeat the washing step. Remove all residual blood cell debris and haemoglobin before proceeding.

**NOTE:** At this point of the procedure, the probes can be stored safely.

**7.4** Remove the wash solution and add to each well **100 µL of permeabilization solution**. Mix well but gently, incubate for **20 minutes at room temperature** and proceed to step **8.** for the click reaction.

### 8. EdU detection

**8.1** Prepare the click assay cocktail in the same order as described in **table 5**. If the ingredients are not added in the order listed, the reaction will not proceed optimally or might even fail.

**Important**: Once the assay cocktail is prepared, use it immediately, at least within the next 15 minutes!

**Table 5:** Click assay cocktails

| Material | Component | Number of well plates | | |
|---|---|---|---|---|
| | | 1 | 2 | 4 |
| **Reaction buffer** | **C- orange** | 9.447 mL | 18.89 mL | 37.78 mL |
| **Reactor system** | **D - green** | 440 µL | 880 µL | 1760 µL |
| **Dye Azide** | **B - red** | 23 µL | 46 µL | 92 µL |
| **Buffer additive (prepared in 4.1.2)** | **E - blue** | 1.10 mL | 2.20 mL | 4.40 mL |
| **Total Volume** | - | **11.01 mL** | **22.02 mL** | **44.04 mL** |

**8.2** Remove permeabilization solution from step 7.4 and add 100 µL of the click assay cocktail to each well and mix well but gently to distribute the assay solution evenly.

**8.3** Incubate the click assay mixture for 30 minutes at room temperature. Protect from light!

**8.4** From the 10x rinse solution prepare a 1x rinse solution by applying following table (table 6). Add the appropriate amount of PBS (1x) (see **table 6**) to the **component F** and mix well (To prevent crystallization, keep component F at room temperature at all times. If component F has crystalized, please warm up to dissolve again. Please see also "cautions"). This additional wash step with this special rinse buffer reduces unspecific, cell number dependent background signal. After use, store any remaining solution at RT. When stored as directed, this stock solution is stable for up to 6 months.

**Table 6:** Amounts of aqueous solution needed to dissolve the rinse buffer to the final work solution

| EdU DetectPro HTS Kit | Volume of 10x rinse buffer | Dilution volume of 1x PBS |
|---|---|---|
| **1 x wellplate** | 2.9 mL | 26.1 mL |
| **2 x wellplates** | 5.8 mL | 52.2 mL |
| **4 x wellplates** | 11.5 mL | 103.5 mL |

Remove Click assay cocktail and wash the cells in each well twice with 150 µL with the 1x rinse solution prepared above.

**8.5** Remove rinse solution. 100 µL of 1 % BSA in PBS is then given to the cells in each well.

**8.6** If performing antibody surface or intracellular labeling, proceed immediately to step **9**, otherwise continue to step **10**.

### *9. Staining intracellular or surface antigens (optional)*

**9.1** Add antibodies against intracellular antigens or against surface antigens that use RPE, PR-tandem or Quantum Dot antibody conjugates. Mix well.

**9.2** Incubate the cells for the time and temperature required for antibody staining. Protect from light.

**9.3** Wash each well twice with 100 µL permeabilization solution. Remove the solution. Add again 100 µL of 1 % BSA in PBS to the cells.

**9.4** Proceed with step **10** for analysing the cells.

**10. Imaging and analysis**

10.1 Close the 96 well plate by using a sealing film, if desired.

10.2 Fluorescence is quantified by scanning the plate using an automated imaging platform equipped with filters appropriate for the dye used. Images of each well can be taken by microscopy.

The excitation and emission maxima of the available dyes are listed in **table 7**.

**Table 7:** Emission and excitation maxima of the available dyes.

| Product number | Dye | Excitation (nm) | Emission (nm) | Filter |
|---|---|---|---|---|
| **BCK-EdUPro-HTS488-200**<br>---------------------<br>**BCK-EdUPro-HTS488-400** | Eterneon$^2$ GREEN Azide<br>(Enhancer system – incl. FITC alternative) | 496 | 516 | Green |
| **BCK-EdUPro-HTS555-200**<br>---------------------<br>**BCK-EdUPro-HTS555-400** | Eterneon$^2$ YELLOW Azide<br>(Enhancer system – incl. Cy3 Azide alternative) | 546 | 579 | Yellow |