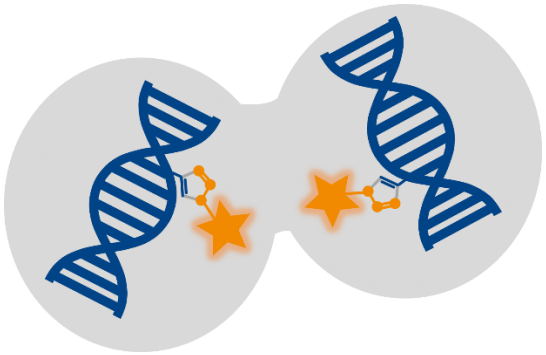
User Manual

Sensitive EdU Cell Proliferation Kit for Flow Cytometry



Version 2.1

Ordering information

(for detailed kit content see **Table 2**)

EdU DetectPro Flow Cytometry Kits:

Product number	EdU	Used fluorescent dye
BCK-EdUPro-FC488	5 mg	Eterneon² GREEN Azide (Enhancer system – incl. FITC alternative)
BCK-EdUPro-FC647	5 mg	Eterneon² RED Azide (Enhancer system – incl. Cy5 alternative)

The *EdU DetectPro FC Kit* contains chemicals to perform 50 reactions (500 µL each).

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

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EdU DetectPro Flow Cytometry 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.

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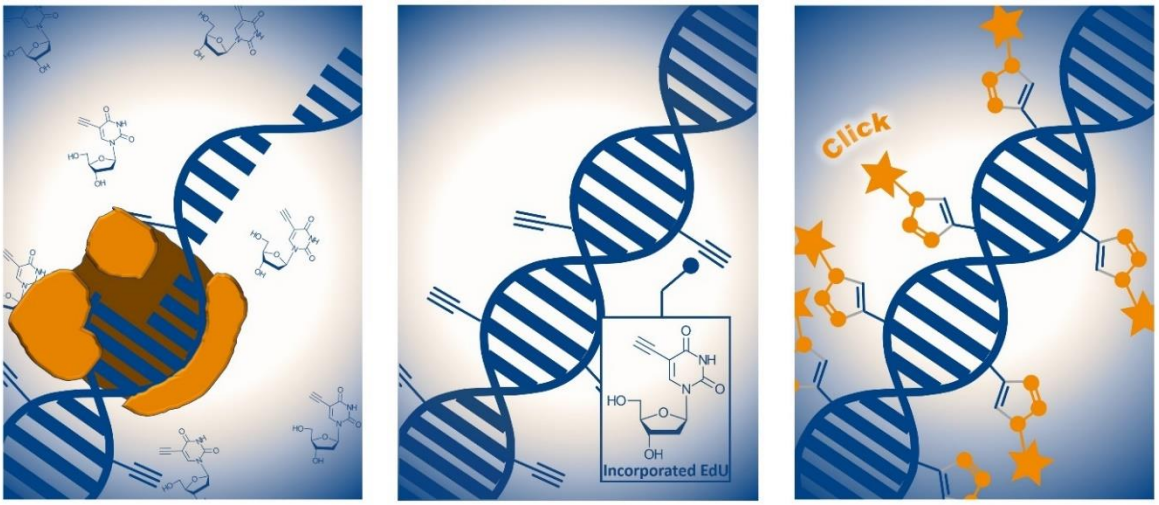
Please read the material safety data sheets (MSDS) provided for each product/component.

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

How the **enhanced EdU DetectPro Flow Cytometry Kit** 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.

This **enhanced *EdU DetectPro*** assay is combining all the above advantages of the standard EdU assay, with higher sensitivity and brightness (signal-to-noise ratio) due to the novel enhancer system in the kit and the unprecedented low level of background.

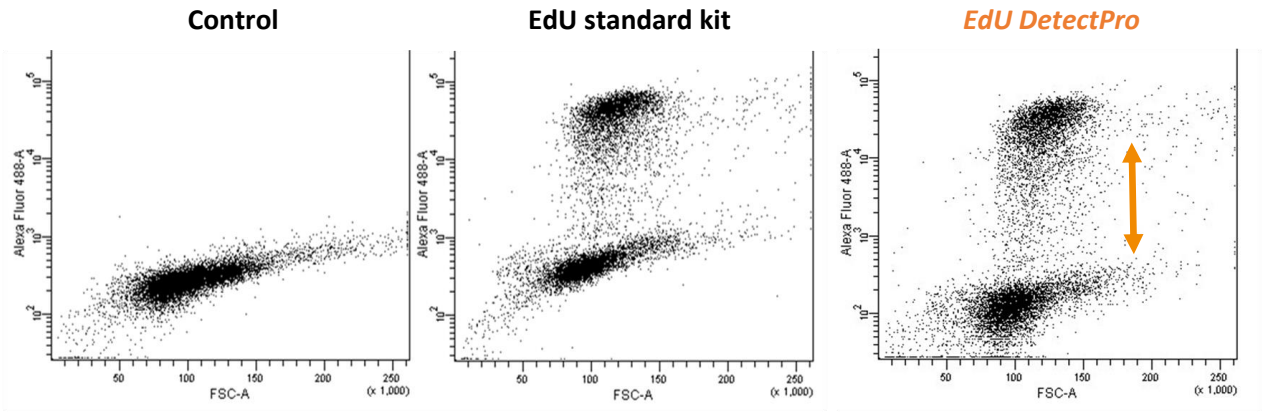


Figure 1: Incubation of Molm13 cells for 2 h with EdU, and subsequent detection of cell proliferation. The standard EdU labeling kit and the enhanced BCK-EdUPro kit were used for comparison; Alexa Fluor 488 voltage setting was adjusted according to the fluorescence signal of the control population.

Standard flow cytometry methods are used to determine the percentage of S-phase cells in the population (**Figure 2**).

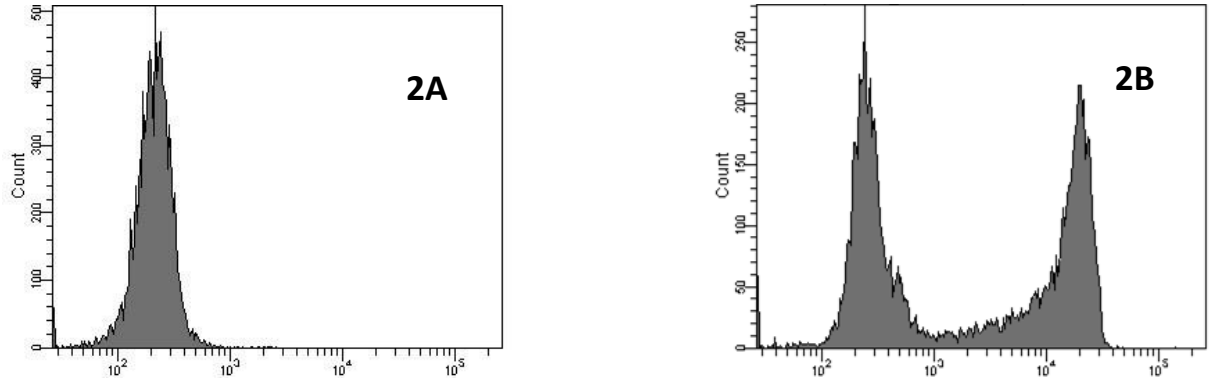


Figure 2: Fluorescence histograms of EdU-incorporation with baseclick EdU DetectPro Flow Cytometry Kit.

Samples of Molm13 cells treated without (**2A**) or with EdU (**2B**) were incubated with 10 µM EdU for 2 hours. The click reaction using **Eterneon² GREEN Azide** was performed according to the recommended staining protocol. Fluorescence intensity of 10.000 cells was measured by flow cytometry. The results are presented in form of histograms, showing the cell number in the y-axis and the Alexa Fluor 488-fluorescence in the x-axis. Alexa Fluor 488 voltage setting was adjusted according to the fluorescence signal of the negative cell population.

2A represents the negative control of proliferating and non-proliferating cells without EdU incorporation. **2B** shows non-proliferating cells without EdU incorporation (left peak) and proliferating cells (S phase) which have incorporated EdU and are labelled with **Eterneon² GREEN Azide** (right peak).

The baseclick *EdU DetectPro Flow Cytometry 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.

Cautions:

DMSO (**Component C**): is known to facilitate the entry of organic molecules into tissues. DMSO is combustible. Handle reagents containing DMSO using equipment and practices appropriate for the hazards posed by such materials. Dispose of the reagents in compliance with all related local arrangements.

Fixative solution (**Component F**): contains paraformaldehyde, which is harmful. Use with appropriate precautions.

Saponin based permeabilization and wash reagent (**Component E**): contains sodium azide, which is highly toxic and yields the extremely toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumping. This solution is orange.

1. Materials provided with the Kit and storage conditions

Table 2: Contents of the kit and storage conditions

Vial-label	Amount	Component	Component long term storage	Kit short term storage *
Component A yellow	10 mg	5-Ethynyl-deoxyuridine (5-EdU)	– 20 °C	2 - 8 °C Dark Do not freeze Dry
Component B red	60 µL	• Eterneon^2 GREEN Azide (BCK-EdUPro-FC488) • Eterneon^2 RED Azide (BCK-EdUPro-FC647)	– 20 °C dark	
Component C purple	5 mL	DMSO	RT	
Component D green	2 mL	Reactor system	2 - 8 °C	
Component E	50 mL	Saponin-based reagent (10x solution)	2 - 8 °C	
Component F	5 mL	Fixation solution (4% Paraformaldehyde)	2 - 8 °C	
Component G blue	400 mg	Buffer additive	2 - 8 °C/ – 20 °C**	
Component H orange	2 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 G 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

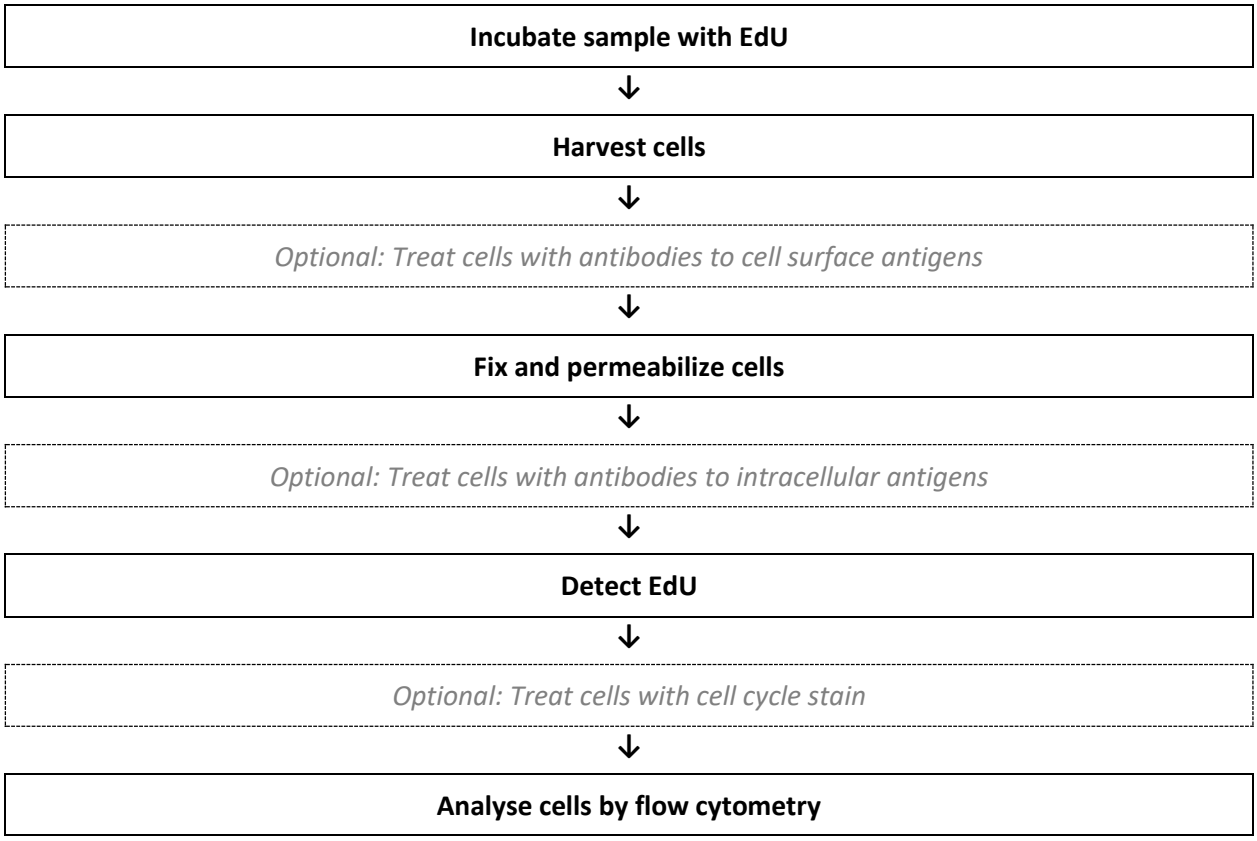
- Reaction tubes (size depends on the volume of reaction cocktail needed)
- Buffered saline solution, such as PBS, D-PBS or TBS
- Appropriate cell culture medium
- 1% BSA (bovine serum albumin) in PBS, pH 7.1 - 7.4
- Deionized water or 18 MΩ purified water
- Flow cytometry tubes

3. Workflow

The following protocol was developed using an 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 Flow Cytometry Assay*



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 A):**

Add 4 mL of DMSO (**Component C**) 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 G):**

Add 4 mL of deionized water to each of the **Component G** 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 3 - 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 freeze and thaw cycles!

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

To prepare 500 mL of saponin-based solution add 450 mL of PBS 1x to 50 mL of saponin 10x solution. A smaller amount can be prepared by diluting a volume of **Component E** with PBS 1x 1:10.

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 - 2 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 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.

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

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

6.1. Wash cells with 3 mL of 1% BSA in PBS. Centrifuge to pellet cells and remove supernatant. If your reaction vial cannot contain 3 mL of 1% BSA in PBS less volume can be used, but not less than 1 mL.

6.2. Dislodge the pellet and resuspend cells in 1% BSA in PBS at 1 x 10^7 cells/mL.

6.3. Add 100 µL of cell suspension or whole blood sample to flow tubes.

6.4. Add surface antibodies and mix well.

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 a saponin-based permeabilization step, but it is also amenable to other cell fixation/permeabilization reagents optimized for your cells. The saponin-based permeablization can be used with cell suspensions containing red blood cells or whole blood as well as with cell suspensions containing different cell types. The morphological light scatter characteristics of leukocytes are maintained by the permeabilization reagent while red blood cells are lysed.

7.1. Remove the incubation media and wash the cells with 3 mL of 1% BSA in PBS. Pellet the cells and remove the supernatant. If your reaction vial cannot contain 3 mL of 1% BSA in PBS less volume can be used, but not less than 1 mL**.**

7.2. Dislodge the cell pellet. Add 100 µL of the fixation solution **(Component F)** to the cells. Mix well and incubate for 15 minutes at room temperature. Protect from light.

7.3. Remove the fixation solution and wash the cells with 3 mL of 1% BSA in PBS. Pellet the cells and remove the supernatant. If your reaction vial cannot contain 3 mL of 1% BSA in PBS less volume can be used, but not less than 1 mL. 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.

7.4. Dislodge the cell pellet. Resuspend the cells in 100 µL of 1x saponin-based permeabilization buffer in PBS (prepared in **4.4**). Mix well, incubate for 20 minutes and proceed to step **8.** for the click reaction.

8. EdU detection

8.1. Prepare the assay cocktail in the same order as described in **Table 3**. 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 3: Click assay cocktails

Material	Component Number	Number of assays			
		1	2	5	10
Deionized water	*Not provided!*	379 µL	758 µL	1895 µL	3790 µL
Reaction buffer (10x)	**Comp. H**	50 µL	100 µL	250 µL	500 µL
Reactor solution	**Comp. D**	20 µL	40 µL	100 µL	200 µL
Dye Azide	**Comp. B**	1 µL	2 µL	5 µL	10 µL
Buffer additive (10x) (prepared in 4.3)	**Comp. G**	50 µL	100 µL	250 µL	500 µL
Total Volume	-	**500 µL**	**1 mL**	**2.5 mL**	**5 mL**

8.2. Add the appropriate amount of the assay cocktail to the cells and mix well to distribute the assay solution evenly.

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

8.4. Wash the cells with 3 mL of 1x Saponin based permeabilization and wash reagent. If your reaction vial cannot contain 3 mL less volume can be used, but not less than 1 mL. Pellet the cells and remove the supernatant. Dislodge the cell pellet. If proceeding with intracellular antibody labeling in step **9**, resuspend the cells in 100 µL of 1x Saponin based permeabilization and wash reagent Otherwise, add 500 µL of 1x Saponin based permeabilization and wash reagent and proceed with step **10** for analyzing the cells with a flow cytometer.

Important: Keep the samples protected from light during the whole procedure.

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 tubes for the time and temperature required for antibody staining. Protect from light.

9.3. Wash each tube with 3 mL of 1x Saponin based permeabilization and wash reagent (prepared in **4.4**). If your reaction vial cannot contain 3 mL less volume can be used, but not less than 1 mL. Pellet the cells and remove the supernatant. Dislodge the cell pellet and resuspend the cells in 500 µL of 1x Saponin based permeabilization and wash reagent.

9.4. Proceed with step **10** for analyzing the cells with a flow cytometer.

10. Imaging and analysis

Use a low flow rate during acquisition, if a traditional flow cytometer with a hydrodynamic focusing is used to measure the total DNA content. The same collection rate and cell concentration should be used for each sample within an experiment. Detect the fluorescent signal generated by DNA content stains with linear amplification. The fluorescent signal generated by EdU labeling is best detected with logarithmic amplification.

The Excitation and emission maxima of the available dyes are listed in **Table 4**.

Table 4: Emission and excitation maxima of the available dyes.

Product number	Dye	Excitation (nm)	Emission (nm)	Filter
BCK-EdUPro-FC488	Eterneon^2 GREEN Azide (Enhancer system – incl. FITC alternative)	496	516	**green**
BCK-EdUPro-FC647	Eterneon^2 RED Azide (Enhancer system – incl. FITC alternative)	643	662	**red**