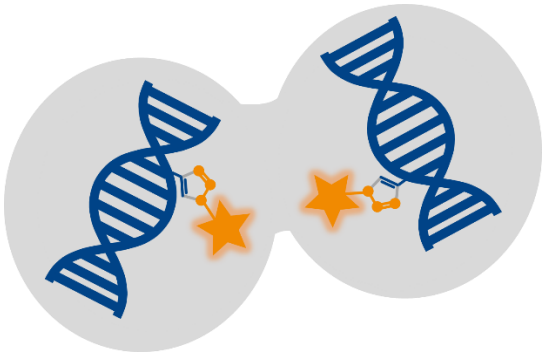
User Manual

ClickTech EdU T Cell Proliferation Kit for Flow Cytometry



Version 2.0

Ordering information

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

ClickTech EdU T Cell Proliferation Kit for 48 reactions:

Product number	20X EdU in PBS	Used fluorescent dye
BCK-TCell-FC488_48	500 µL	Eterneon² GREEN Azide (Enhancer system – incl. FITC alternative)
BCK-TCell-FC555_48	500 µL	Eterneon² YELLOW Azide (Enhancer system – incl. 5-TAMRA alternative)
BCK-TCell-FC647_48	500 µL	Eterneon² RED Azide (Enhancer system – incl. Cy5 alternative)

ClickTech EdU T Cell Proliferation Kit for 192 reactions:

Product number	20X EdU in PBS	Used fluorescent dye
BCK-TCell-FC488_192	2 mL	Eterneon² GREEN Azide (Enhancer system – incl. FITC alternative)
BCK-TCell-FC555_192	2 mL	Eterneon² YELLOW Azide (Enhancer system – incl. 5-TAMRA alternative)
BCK-TCell-FC647_192	2 mL	Eterneon² RED Azide (Enhancer system – incl. Cy5 alternative)

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

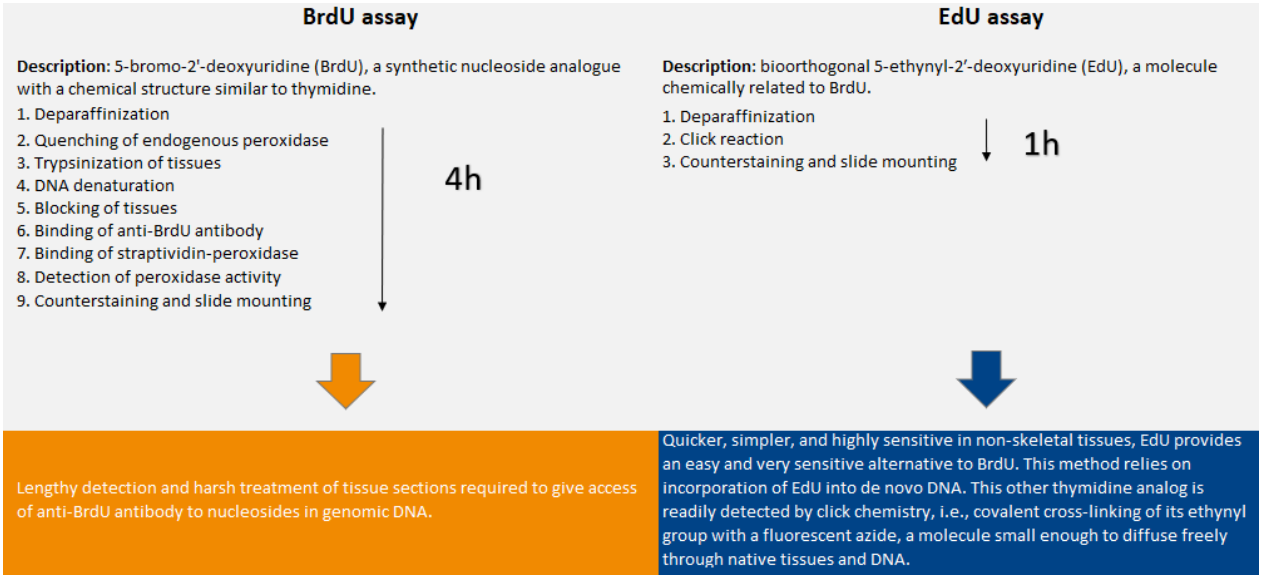
online: www.baseclick.eu
orders: orders@baseclick.eu
support: support@baseclick.eu
phone: +49 89 9699 3401
fax: +49 89 9699 4696

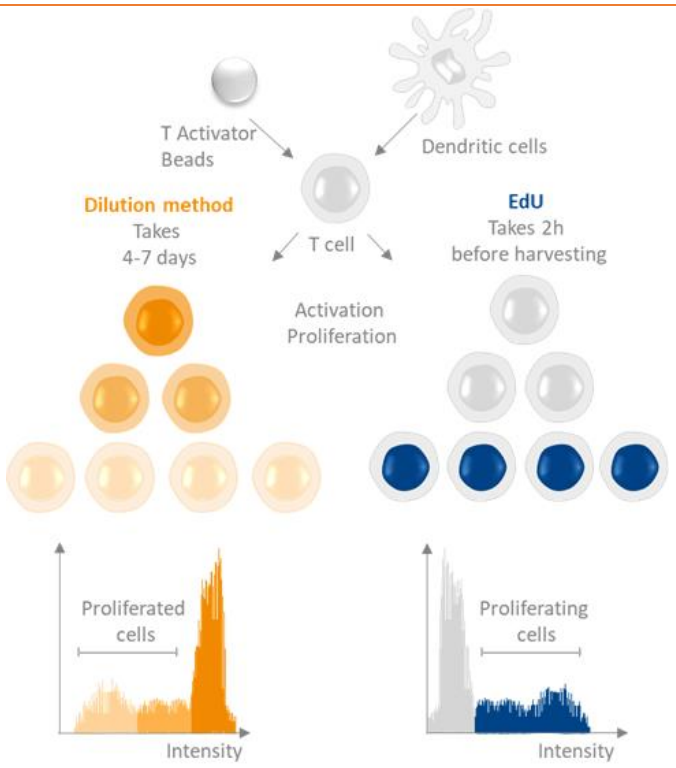
ClickTech EdU T Cell Proliferation Kit

Introduction and product description:

The detection of T cell proliferation is of utmost importance in immunology research, as it is a major indicator of T cell activation. There are two methods detecting T Cell proliferation:
a) feeding of cells with nucleoside analogues feeding (2 h incubation of cells)
b) fluorescent cell tracing reagents (4-7 days incubation of cells)

a) The used BrdU assays have several limitations as it is time consuming (4 h) and it requires harsh, denaturing conditions of tissue to allow anti-BrdU antibodies to reach the genomic DNA. Additionally the low sensitivity requires a high number of target cells.
b) The indirect fluorescent cell tracing reagent method depends on long incubation time and this approach exhibits several limitations as it can reduce T cell proliferation, viability, responsiveness, and cell proliferation can only be determined after completion of full rounds of cell division.
A superior alternative to both of these methods is the incorporation of the detectable nucleoside analogue 5-Ethynyl-2'deoxyuridine (EdU) The major advantages of our ClickTech EdU T cell proliferation assay are that this method is less cytotoxic, lower in workload (1h), it offers superior signal-to-background ratio and allows for improved detection of interferon gamma responses as well as multiplexing.





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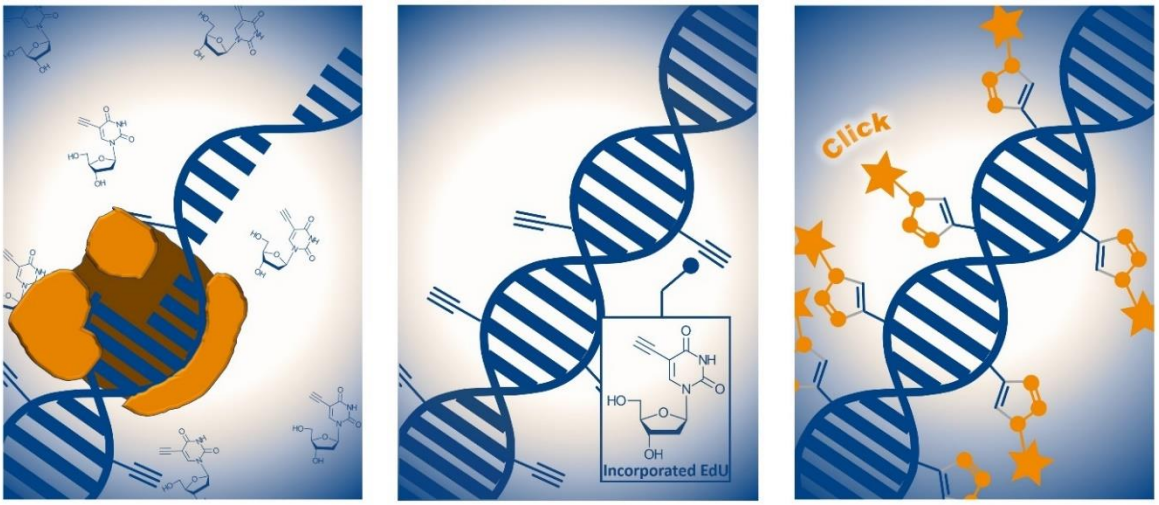
Please read the material safety data sheets (MSDS) provided for each product/component.

Literature Citation: When describing a procedure for publication using this product, please refer to it as *baseclick ClickTech EdU T Cell Croliferation Kit*.

How the **ClickTech EdU T cell proliferation** assay works



The baseclick *ClickTech EdU T cell proliferation Kit* overcomes the limitations of other assays, thus providing a superior alternative for measuring T cell proliferation.

Just as in the traditional EdU proliferation kits from baseclick, also here EdU (5-ethynyl-2'-deoxyuridine) (a thymidine analog) is incorporated into DNA during active DNA synthesis.

The simple click chemistry detection procedure is complete within 1h and is compatible with multiplexing for content and context-rich results.

The baseclick *ClickTech EdU T Cell Proliferation Kit* can be combined with antibody staining for 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
Fixable Viability Dyes (e.g. eFluor™780)	Compatible

* Compatibility indicates which of the involved components are unaffected 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 generally good fluorescence for rabbit and chicken anti-GFP and very low fluorescence for mouse monoclonal antibodies. This should be understood as a general guideline, but results may still greatly vary depending on the individual chosen antibody.

Cautions:

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

Saponin based permeabilization and wash reagent (**Component P**): 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 plumbing. 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 48 Assays	Amount 192 Assays	Component	Component long term storage	Kit storage*
Component E yellow	500 µL	2 mL	5-Ethynyl-deoxyuridine (5-EdU) (20x)	– 20 °C	2 - 8 °C Dark Do not freeze Dry
Component D red	5 µL	25 µL	• Eterneon² GREEN Azide (BCK-TCell-FC488) • Eterneon² YELLOW Azide (BCK-TCell-FC555) • Eterneon² RED Azide (BCK-TCell-FC647)	– 20 °C dark	
Component C green	25 µL	100 µL	Reactor system	2 - 8 °C	
Component P	2 mL	6 mL	Saponin-based reagent (10x solution)	2 - 8 °C	
Component F	5 mL	4x 5 mL	Fixative solution (4% Paraformaldehyde)	2 - 8 °C	
Component B blue	5 mg	4x 5 mg	Buffer additive	2 - 8 °C/ – 20 °C**	
Component RB orange	100 µL	500 µL	Reaction buffer (10x)	2 - 8 °C	

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

** When dissolved the component B 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.

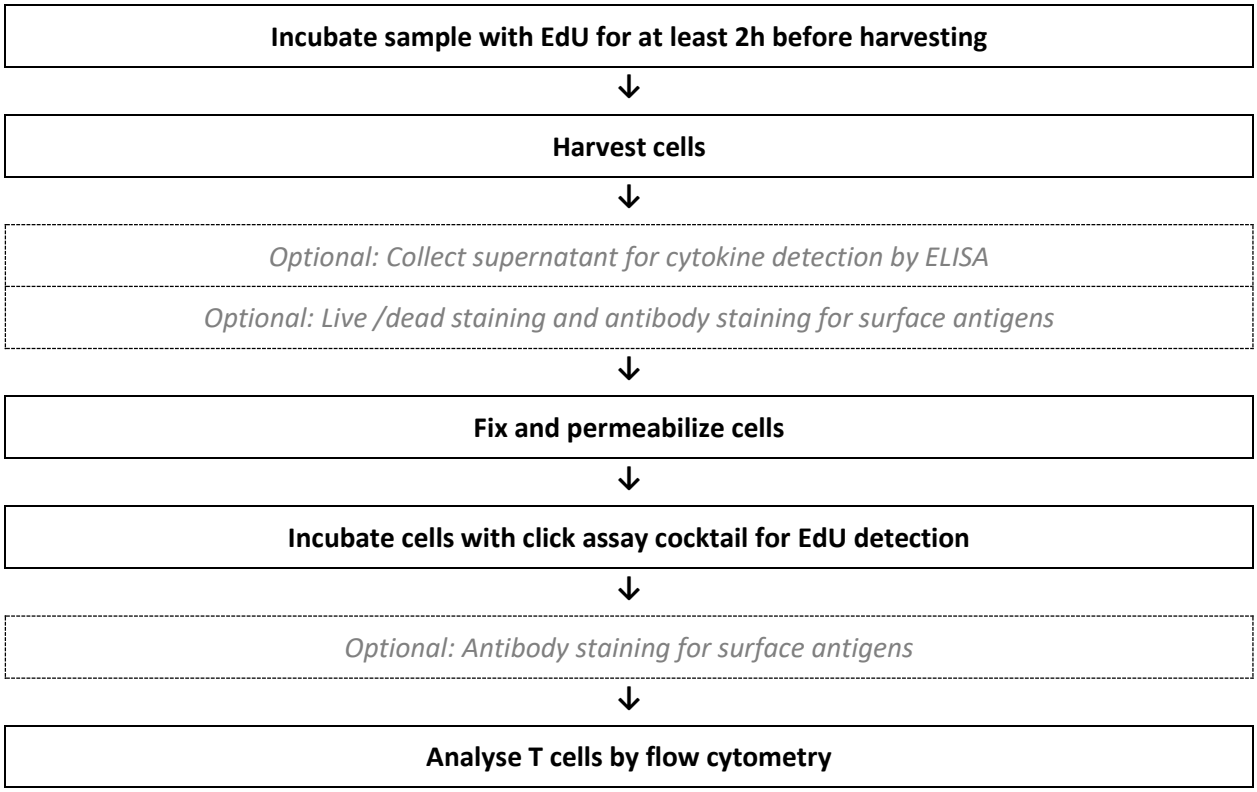
2. Required Material and Equipment not included in this Kit

- Reaction tubes (size depends on the volume of reaction cocktail needed)
- Buffered saline solution, such as PBS
- Appropriate cell culture medium
- 1% BSA (bovine serum albumin) in PBS
- Deionized water or 18 MΩ purified water
- 96 well plates for non-adherent cultures

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 and cell density. We recommend to set up allogeneic cocultures by preparing 1:10 mixtures of Monocyte-derived dendritic cells (moDCs) and peripheral blood leukocytes (PBLs) in 100 µl in a 96-well plate for non-adherent cultures. Appropriate positive and negative controls for T cell activation should be set up. Samples containing either only moDCs or only PBLs may be used as negative controls. As positive control, stimulation of PBLs with T cell activator beads (e.g., Anti-CD3/CD28 beads).

Workflow scheme for the *EdU T cell 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 10x stock solution of the buffer additive (Component B):**

Add 50 µL of deionized water to one of the **Component B** (5 mg) 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!

4.3. **Dilution of the saponin based reagent (10x solution) (Component P):**

To prepare 50 mL of 1x saponin-based solution add 45 mL of PBS to 5 mL of 10x saponin solution or to prepare 20 mL of 1x saponin-based solution add 18 mL of PBS 1x to 2 mL of saponin. Smaller amounts of 1x saponin-based solution can be prepared by diluting **Component P** 1:10 in PBS.

5. Labelling 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 (2h before harvesting).

5.2. Dilute **Component E** (EdU 200µM) with your cell culture medium at a 1:10 ratio to get a 20 µM EdU solution and equilibrate it to 37°C. Add 100 µl (one volume) of the equilibrated EdU-containing medium to each well and incubate for minimum 2 h. We recommend using a concentration of 10 µM for 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. Harvest cells. If performing antibody labelling, proceed to step **6**, otherwise continue to step **7**.

6. *Antibody staining (optional)*

6.1. Wash cells with 100 µL/per well of 1% BSA in PBS.

6.2. Add antibodies and mix well (for CD3 receptor e.g. mouse anti-CD3 APC-conjugated antibody)

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

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

6.4. Wash cells with 100 µL/per well of 1% BSA in PBS

6.5. 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 permeabilization 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 100 µL of 1% BSA in PBS. Pellet the cells and remove the supernatant.

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

7.3. Remove the fixative solution and wash the cells twice with 150 µL of 1% BSA in PBS. Remove the supernatant.

7.4. Resuspend the cells in 100 µL of 1x saponin-based permeabilization buffer in PBS (prepared in **4.3**). Mix well, incubate for 20 minutes on ice 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			
		10	50	100	200
Deionized water	*Not provided!*	75 µL	375 µL	750 µL	1500 µL
Reaction buffer (10x)	**Comp. RB**	10 µL	50 µL	100 µL	200 µL
Reactor system	**Comp. C**	4 µL	20 µL	40 µL	80 µL
Dye Azide	**Comp. D**	1 µL	5 µL	10 µL	20 µL
Buffer additive (10x) (prepared in 4.2)	**Comp. B**	10 µL	50 µL	100 µL	200 µL
Total Volume	-	**100 µL**	**500 µL**	**1 mL**	**2 mL**

8.2. Incubate the cells for 1 hour with 10 µL of the assay cocktail and mix well to distribute the assay solution evenly. Protect from light! **Note**: higher click assay volumes may be used for shorter incubation times.

8.3. Wash the cells with 200 µL of 1x saponin-based solution. If proceeding with antibody labelling in step **9**, resuspend the cells in 100 µL of 1x saponin-based solution. Otherwise, proceed with step **10** for flow cytometry analysis.

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 the cells with 200 µL of 1x saponin-based solution and afterwards twice with 100 µL PBS. Proceed with step **10** for flow cytometry analysis.

10. Flow cytometry analysis

Use a low flow rate during acquisition, if a traditional flow cytometer with a hydrodynamic focusing is used to measure the 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 dyes 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)
BCK-Tcell-FC488	Eterneon^2 GREEN Azide (Enhancer system – incl. FITC alternative)	496	516
BCK-Tcell-FC555	Eterneon^2 YELLOW Azide (Enhancer system – incl. TAMRA alternative)	546	579
BCK-Tcell-FC647	Eterneon^2 RED Azide (Enhancer system – incl. Cy5 alternative)	643	662