



EdU HTS Kit

User Manual



Ordering information:

(for detailed kit content see Table 2)

EdU HCS Kits ready for 2 x 96well plate assays:

Product number	20X EdU in PBS	Used fluorescent dye
BCK-HTS488-2	2 mL	6-FAM Azide ($\lambda_{\text{abs}}=496/\lambda_{\text{em}}=516$)
BCK-HTS555-2	2 mL	5-TAMRA-PEG3-Azide ($\lambda_{\text{abs}}=546/\lambda_{\text{em}}=579$)
BCK-HTS594-2	2 mL	5/6-Sulforhodamine 101-PEG3-Azide ($\lambda_{\text{abs}}=584/\lambda_{\text{em}}=603$)
BCK-HTS647-2	2 mL	Cyanine 5 Azide ($\lambda_{\text{abs}}=646/\lambda_{\text{em}}=662$)

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

EdU HCS Kits ready for 4 x 96well plate assays:

Product number	20X EdU in PBS	Used fluorescent dye
BCK-HTS488-4	4 mL	6-FAM Azide ($\lambda_{\text{abs}}=496/\lambda_{\text{em}}=516$)
BCK-HTS555-4	4 mL	5-TAMRA-PEG3-Azide ($\lambda_{\text{abs}}=546/\lambda_{\text{em}}=579$)
BCK-HTS594-4	4 mL	5/6-Sulforhodamine 101-PEG3-Azide ($\lambda_{\text{abs}}=584/\lambda_{\text{em}}=603$)
BCK-HTS647-4	4 mL	Cyanine 5 Azide ($\lambda_{\text{abs}}=646/\lambda_{\text{em}}=662$)

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

EdU HCS Kits ready for 20 x 96well plate assays:

Product number	20X EdU in PBS	Used fluorescent dye
BCH-HTS488-20	20 mL	6-FAM Azide ($\lambda_{\text{abs}}=496/\lambda_{\text{em}}=516$)
BCH-HTS555-20	20 mL	5-TAMRA-PEG3-Azide ($\lambda_{\text{abs}}=546/\lambda_{\text{em}}=579$)
BCH-HTS594-20	20 mL	5/6-Sulforhodamine 101-PEG3-Azide ($\lambda_{\text{abs}}=584/\lambda_{\text{em}}=603$)
BCH-HTS647-20	20 mL	Cyanine 5 Azide ($\lambda_{\text{abs}}=646/\lambda_{\text{em}}=662$)

The Kit contains sufficient material for 20 x 96 well plate assays

To place your order, please contact us under:

- phone: +49 8158 903867
- fax: +49 8158 903894
- email: orders@baseclick.eu

EdU 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 analogs like [³H]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 [³H]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 *EdU HTS Kits* overcome these limitations, providing a superior alternative to BrdU and [³H]thymidine assays for directly measuring DNA synthesis of adherent cells in 96 well plates. 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 HTS Assays* are not antibody based and therefore do not require DNA denaturation for detection of the incorporated nucleoside. Instead, the *EdU HTS Kits* 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.

For research use only.

Information in this document is subject to change without notice. baseclick GmbH assumes no responsibility for any errors that may appear in this document.

baseclick GmbH disclaims all warranties with respect to this document, expressed or implied, including but not limited to those of merchantability or fitness for a particular purpose. In no event shall baseclick GmbH be liable, whether in contract, tort, warranty, or under any statute or on any other basis for special, incidental, indirect, punitive, multiple or consequential damages in connection with or arising from this document, including but not limited to the use thereof.

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 HTS Kit*.

The baseclick *EdU 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). Not all GFP antibodies recognize the same antigen site. Rabbit and chicken anti-GFP antibodies result in a good fluorescent amount. The mouse monoclonal antibodies tested are not recommended for this application because they do not generate an acceptable amount of fluorescence.

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	Amount for 20 assays/well plates	Component	Component long term storage	Kit storage
Component A yellow	2 mL	4 mL	20 mL	5-Ethynyl-deoxyuridine (5-EdU)	-20°C	2 - 8°C Dark Do not freeze Dry
Component B red	130 µL	2 x 130 µL	9 x 130 µL	6-FAM Azide 5-TAMRA-PEG3-Azide 5/6-Sulforhodamine 101-PEG3-Azide Cyanine 5 Azide	-20°C dark	
Component C orange	20 mL	40 mL	200 mL	Reaction buffer	RT	
Component D green	1 mL	1 mL	5 mL	Catalyst solution	RT	
Component E blue	200 mg	400 mg	2 g	Buffer additive	- 20°C	
Component F ** grey	6ml	2 x 6ml	58ml	Rinse buffer (10X)	RT	

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

**** 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 which can be downloaded from our webpage www.baseclick.eu

This solution is stored at RT and will crystallize at lower temperatures. In later case 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

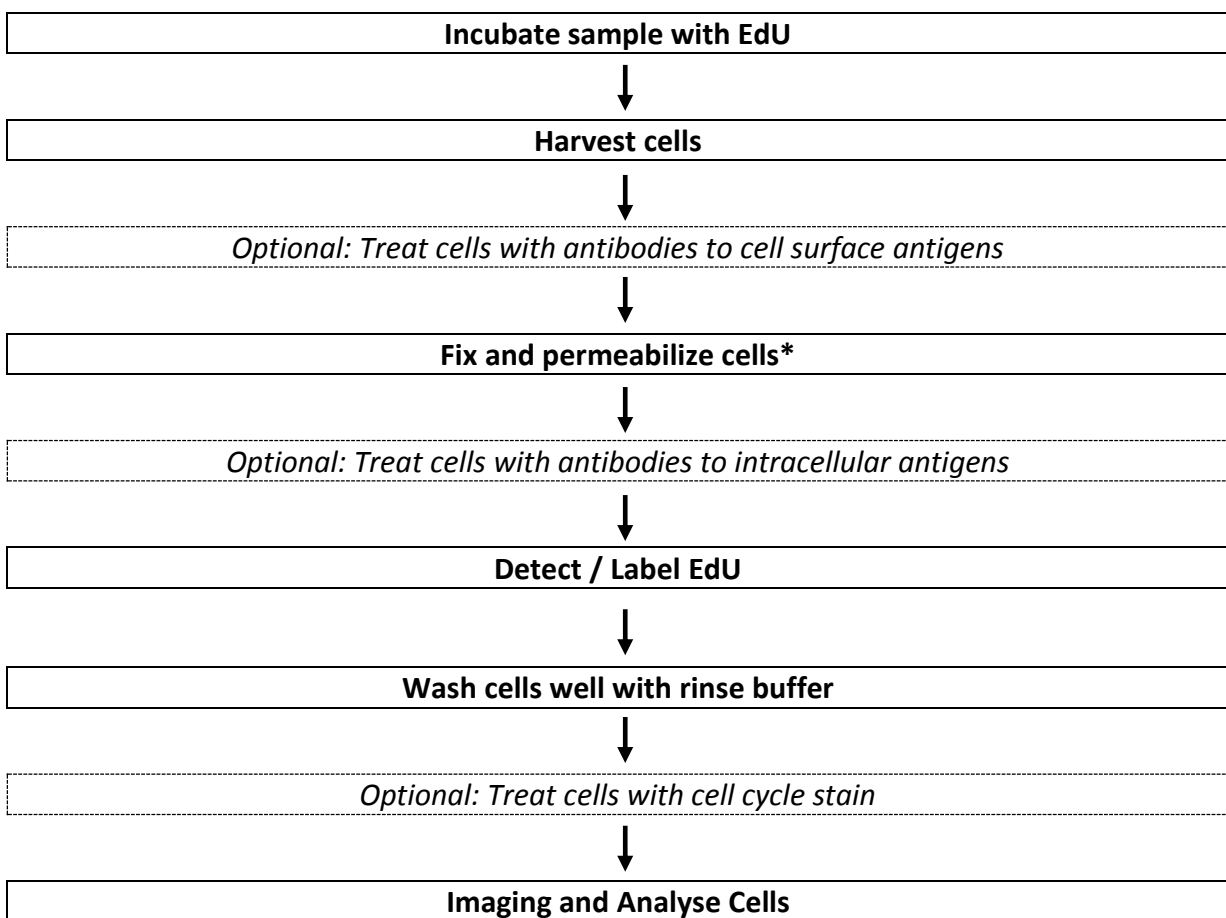
- Adherent cells
- Reaction tubes (size depends on the volume of reaction cocktail needed)
- Buffered saline solution, such as PBS, D-PBS or TBS
- Fixative solution (4% Paraformaldehyde in PBS)
- Saponin-based permeabilization and wash reagent (10x solution)
- Appropriate cell culture medium
- 1% BSA (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 μ M 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 HCS 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 μ M 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 μ M

EdU HCS Kit	20X EdU solution	In dilution Volume for 2X EdU solution in 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
10 x 96 well plates	10 mL	90 mL
20 x 96 well plates	20 mL	180 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 thaw and freeze cycles!

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

EdU HCS Kit	Buffer additive (solide)	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
10 x 96 well plates	1 g	10 mL
20 x 96 well plates	2 g	25 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.
- 5.4 Harvest cells. 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 a saponin-based permeabilization step. The saponin-based permeabilization and wash reagent 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 the permeabilization reagent while red blood cells are lysed.

- 7.1** Remove the incubation media and wash the cells, each well with 100 μ L of 1% BSA in PBS. Afterwards remove the wash solution.
- 7.2** Add 100 μ L 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 μ L of 1% BSA in PBS. Remove the solution. 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 μ L of 1x saponin-based permeabilization buffer in PBS. Mix well but gently 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	10	20
Reaction buffer	C - orange	9.635 mL	19.27 mL	38.54 mL	96.35 mL	192.7 mL
Catalyst solution	D - green	220 μ L	440 μ L	880 μ L	2.2 mL	4.4 mL
Dye Azide (10 mM)	B - red	55 μ L	110 μ L	220 μ L	550 μ L	1.1 mL
Buffer additive (prepared in 4.1.2)	E - blue	1.1 mL	2.2 mL	4.4 mL	11 mL	22 mL
Total Volume	-	11.01 mL	22.02 mL	44.04 mL	110.1 mL	220.2 mL

- 8.2** Remove Wash solution from step 7.4 and add 100 μ L 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. 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 buffer additive to the final work solution

EdU HCS Kit	Volume of 10X rinse buffer	Dilution volume of 1X PBS
1 x wellplate Kit	2.9 mL	26.1 mL
2 x wellplate Kit	5.8 mL	52.2 mL
4 x wellplate Kit	11.5 mL	103.5 mL
10 x wellplate Kit	28.8 mL	259.2 mL
20 well plate Kit	57.6 mL	518.4 mL

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

- 8.5** Remove rinse solution. 100 μ L 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 μ L 1x saponin-based permeabilization and wash reagent. Remove the solution. Add again 100 μ L of 1% BSA in PBS to the cells.
- 9.4** Proceed with step **10** for analyzing 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-HTS488	6-FAM Azide (BCK-FC488)	496	516	Green
BCK-HTS555	5-TAMRA-PEG3-Azide (BCK-FC555)	546	579	Violet
BCK-HTS594	5/6-Sulforhodamine 101-PEG3-Azide (BCK-FC594)	584	603	Orange
BCK-HTS647	Cyanine 5 Azide (BCK-FC647)	646	662	Red

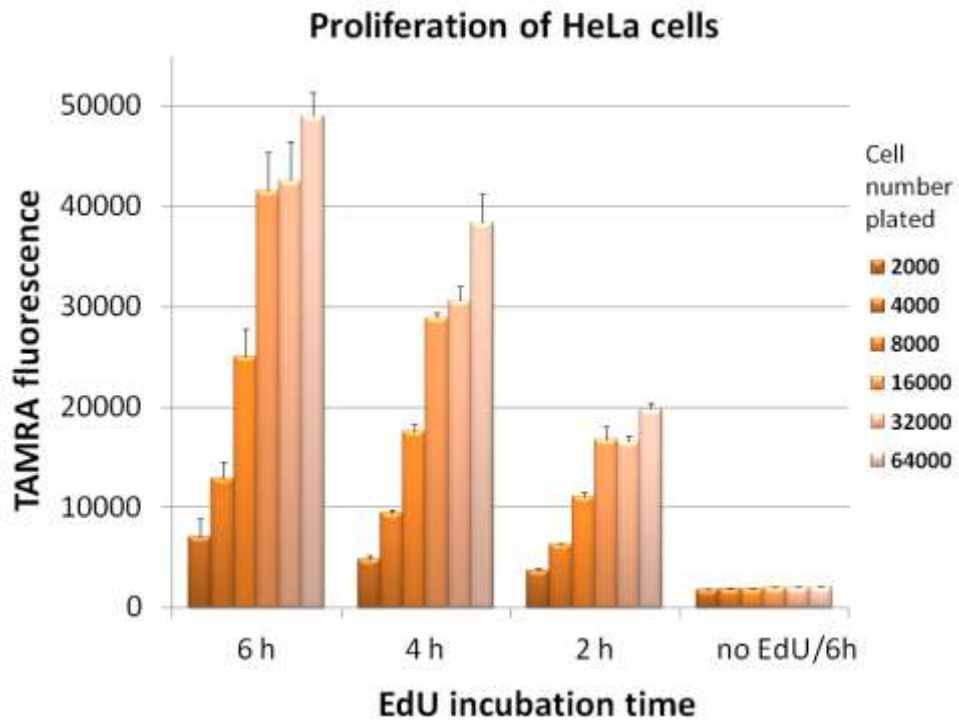
11. Example of the data derived from an EdU HTS Kit based experiment:

Figure 1. Detection of EdU incorporation depending on cell number and EdU incubation time.

HeLa cells were seeded in a transparent 96well cell culture plate with indicated cell numbers per well. After 42 h cells were incubated with or without 10 μ M EdU for 2, 4 or 6 h and subsequently EdU incorporation was detected using the EdU-HTS Assay Kit and a fluorescence plate reader. Mean and SD values from quadruplicates are shown.

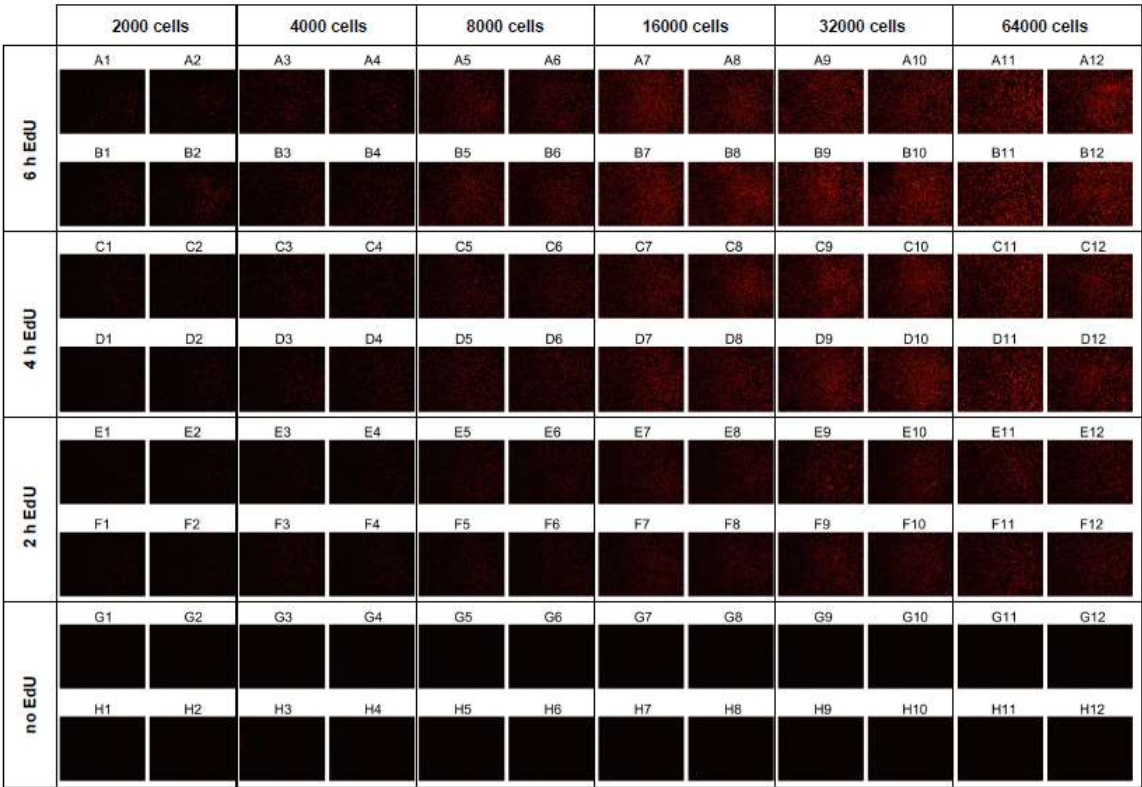


Figure 2: Detection of EdU incorporation via fluorescence microscopy.
A fluorescence photograph (40x) of the center of each 96 well of the, with rinse buffer washed assay plate was captured and presented using the Nikon NIS-elements software.

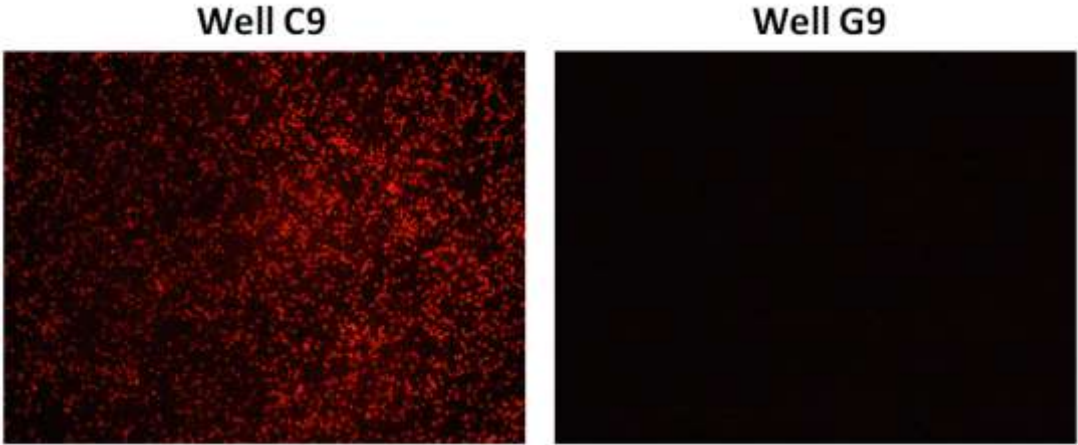


Figure 3: Zoom on the samples after Click reaction and washing (in Figure 5) cells which do EdU proliferation in well C9 and cells, which haven't received EdU, in well G9.



baseclick GmbH

Bahnhofstraße 9-15

82327 Tutzing, Germany

Phone: +49 8158 903867

Fax: +49 8158 903894

Email: info@baseclick.eu