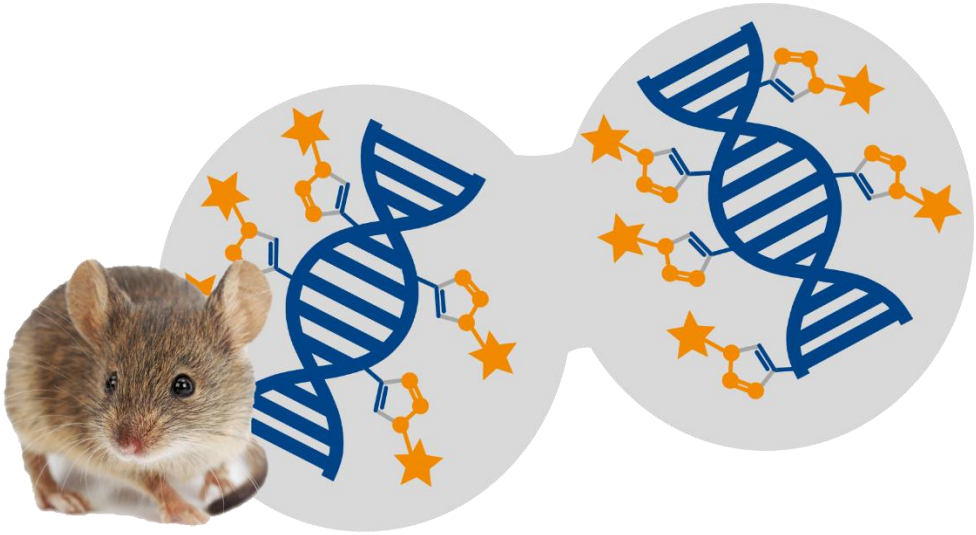
User Manual

EdU in vivo Kits



Version 5.1

Ordering information

EdU *in vivo* Kits for Imaging (IM):

Product number	Basic product	Additional EdU	Dye
BCK488-IV-IM-S	BCK-EdU488	50 mg	6-FAM-Azide
BCK555-IV-IM-S	BCK-EdU555	50 mg	5-TAMRA-PEG3-Azide
BCK594-IV-IM-S	BCK-EdU594	50 mg	5/6-Sulforhodamine 101-PEG3-Azide
BCK647-IV-IM-S	BCK-EdU647	50 mg	Eterneon Red 645 Azide
BCK488-IV-IM-M	BCK-EdU488	500 mg	6-FAM-Azide
BCK555-IV-IM-M	BCK-EdU555	500 mg	5-TAMRA-PEG3-Azide
BCK594-IV-IM-M	BCK-EdU594	500 mg	5/6-Sulforhodamine 101-PEG3-Azide
BCK647-IV-IM-M	BCK-EdU647	500 mg	Eterneon Red 645 Azide
BCK488-IV-IM-L	BCK-EdU488	1000 mg	6-FAM-Azide
BCK555-IV-IM-L	BCK-EdU555	1000 mg	5-TAMRA-PEG3-Azide
BCK594-IV-IM-L	BCK-EdU594	1000 mg	5/6-Sulforhodamine 101-PEG3-Azide
BCK647-IV-IM-L	BCK-EdU647	1000 mg	Eterneon Red 645 Azide

EdU *in vivo* Kits for Flow Cytometry (FC):

Product number	Basic product	Additional EdU	Dye
BCK488-IV-FC-S	BCK-FC488	50 mg	6-FAM-Azide
BCK555-IV-FC-S	BCK-FC555	50 mg	5-TAMRA-PEG3-Azide
BCK594-IV-FC-S	BCK-FC594	50 mg	5/6-Sulforhodamine 101-PEG3-Azide
BCK647-IV-FC-S	BCK-FC647	50 mg	Eterneon Red 645 Azide
BCK488-IV-FC-M	BCK-FC488	500 mg	6-FAM-Azide
BCK555-IV-FC-M	BCK-FC555	500 mg	5-TAMRA-PEG3-Azide
BCK594-IV-FC-M	BCK-FC594	500 mg	5/6-Sulforhodamine 101-PEG3-Azide
BCK647-IV-FC-M	BCK-FC647	500 mg	Eterneon Red 645 Azide
BCK488-IV-FC-L	BCK-FC488	1000 mg	6-FAM-Azide
BCK555-IV-FC-L	BCK-FC555	1000 mg	5-TAMRA-PEG3-Azide
BCK594-IV-FC-L	BCK-FC594	1000 mg	5/6-Sulforhodamine 101-PEG3-Azide
BCK647-IV-FC-L	BCK-FC647	1000 mg	Eterneon Red 645 Azide

EdU *in vivo* Kits for High-throughput Screening (HTS):

Product number	Basic product	Additional EdU	Dye
BCK488-IV-HTS-S	BCK-HTS488	50 mg	6-FAM-Azide
BCK555-IV-HTS-S	BCK-HTS555	50 mg	5-TAMRA-PEG3-Azide
BCK488-IV-FC-M	BCK-HTS488	500 mg	6-FAM-Azide
BCK555-IV-FC-M	BCK-HTS555	500 mg	5-TAMRA-PEG3-Azide
BCK488-IV-FC-L	BCK-HTS488	1000 mg	6-FAM-Azide
BCK555-IV-FC-L	BCK-HTS555	1000 mg	5-TAMRA-PEG3-Azide

Generally, one of the following standard dyes is delivered with the kits:

Dye	Absorption/emission	ε	Analogous dyes
6-FAM-Azide	(λ_{abs}=496/λ_{em}=516)	83.000 $cm^{-1}M^{-1}$	**Alexa 488, DyLight488, FluorX, ATTO488**
5-TAMRA-PEG3-Azide	(λ_{abs}=546/λ_{em}=579)	91.000 $cm^{-1}M^{-1}$	**Alexa 555, Cy3**
5/6-Sulforhodamine 101-PEG3-Azide	(λ_{abs}=584/λ_{em}=603)	116.000 $cm^{-1}M^{-1}$	**Texas Red, Alexa 594, ATTO 594**
Eterneon Red 645 Azide	(λ_{abs}=643/λ_{em}=662)	250.000 $cm^{-1}M^{-1}$	**Alexa 647, Cy5, ATTO647**

The EdU cell proliferation kits are optimized and tested for these standard dyes. If you wish to purchase another dye or azide component, please contact us for price inquiry and suggestions.

Generally, the following standard amounts of EdU are delivered with the kits:

In order to freely choose the right kit for your special *in vivo* application, our *in vivo* kits are designed in a sense that you combine one of our three baseclick EdU cell proliferation kits such as Imaging kit (BCK-IV-IM), flow cytometry kit (BCK-IV-FC) and our high throughput screening kit (BCK-IV-HTS) with the dye of choice and the right EdU content for your animal model.

Depending on your animal or the number of animals to be tested you can choose between three kit sizes S, M and L with increasing EdU content, as indicated in the following table.

Kit size	*Content of EdU*
S	*50 mg*
M	*500 mg*
L	*1000 mg*

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

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EdU in vivo 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 [^{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.

The baseclick *EdU in vivo Kits* overcome these limitations, providing a superior alternative to BrdU and [^{3}H]thymidine assays for directly measuring DNA synthesis *in vivo*. 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 in vivo Kits* are not antibody based and therefore do not require DNA denaturation for detection of the incorporated nucleoside. Instead, the *EdU in vivo Kits* utilize a fast, efficient and reliable method based on the so called "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.

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This is not an allowance to perform *in vivo* studies. Contact your local authorities for the animal testing regulations and authorization of your *in vivo* experiments, if not already done.

Literature Citation: When describing a procedure for publication using this product, please cite it as the *EdU in vivo Kit* from baseclick GmbH.

The baseclick *EdU in vivo Kit* is compatible with several cell cycle dyes and 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
Fluorescent proteins (e.g. GFP)	Use anti-GFP antibodies **before** the EdU detection reaction or use organic dye-based reagents for protein expression detection

Cautions:

EdU: is a nucleoside analogue which can be incorporated into DNA. Handle and dispose EdU in compliance with all pertaining local regulations. EdU is soluble in DMSO, water, alcohol and aqueous buffers. For *in vivo* experiments, EdU can be solved in a buffered saline solution such as PBS.

EdU toxicity: No cytotoxicity could be measured up to 1 mM EdU. The data on the pharmacotoxicity of EdU are in publication process at the moment by baseclick.

The cautions of other ingredients are described in the according User Manual you receive with your kit or can also be downloaded from our website www.baseclick.eu.

MSDS: the appropriate MSDS for all components of each individual kit can be downloaded from our website www.baseclick.eu.

1. Material provided and storage conditions

For the specification of the material provided and their storage conditions please see the specific user manual of your purchased kit which is included in your baseclick box or can be downloaded from our webpage, since each kit has its proper composition.

2. Required Material and Equipment

The required materials and equipment depends on your provided kit; please see the specific kit´s user manual delivered with the kit you purchased.

- Reaction tubes (size depends on the volume of reaction cocktail needed)
- Buffered saline solution, such as PBS, D-PBS or TBS
- Fixation solution (3 - 4% Paraformaldehyde in PBS)*
- Permeabilization solution (for example, 0.5% Triton® X-100 in PBS)*
- 1% BSA (bovine serum albumin) in PBS, pH 7.1 - 7.4
- Deionized water or 18 MΩ purified water

* already included in flow cytometry kits BCK-IV-FC (S, M & L)

3. Preparation of the EdU stock solution

- Allow all vials to warm to room temperature before opening.
- For the preparation of 10 mM EdU solution, dissolve EdU in the appropriate amount of phosphate buffered saline PBS according to **Table 2** 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.

Table 2: Amounts of PBS needed to dissolve EdU for a final concentration of 10 mM.

EdU	Amount of PBS
50 mg	20 mL
100 mg	40 mL
500 mg	200 mL
1 g	400 mL
5 g	2000 mL

The preparations of other stock solutions are described in the according User Manual you get with your kit or can be downloaded from our website www.baseclick.eu.

4. EdU Administration

We recommend testing a range of EdU concentrations to determine the optimal concentration for your experiment. If currently using BrdU-based assays, a similar concentration and incubation time to BrdU is a good starting point for EdU. The optimal concentration may vary depending upon the duration of the pulse. Lower concentrations are recommended for longer incubation time. General recommendations are listed below (See point **5**).

EdU administration to the animals can be performed following injection or media incorporation.

Successful EdU-labeling in animals has been reported for the following species:

Mouse: Salic A et al., *Proc Natl Acad Sci USA* **2008**, *105*, 2415-2420.

Rat: Guo J. et al, *Cardiovascular Diabetology* **2012**, *11*, 150.

Nematode (*C. elegans*): Dorsett M, Westlund B, Schedl T, *Genetics* **2009**, *183*, 233-247.

Cricket (Gryllus bimaculatus): Bando T, Mito T, Maeda Y et al., *Development* **2009**, *136*, 2235-2245.

Chicken (Gallus domesticus): Kaiser CL, Kamien AJ, Shah PA, *Laryngoscope* **2009**, *119*, 1770-1775.

Zebra Fish larva (*Danio rerio*): Luedtke W. et al., *PNAS* **2011**, *108*, 51.

5. General recommendations for different animals

5.1 Mouse / Rat: see page **8**.

5.2 Nematode (C. elegans): see page **9**.

5.3 Cricket (Gryllus bimaculatus): see page **9**.

5.4 Chicken (Gallus domesticus): see page **10**.

5.1 Mouse / Rat:

EdU can be injected in different doses from 10 - 200 mg/kg. Using 50 mg/kg of EdU in PBS i.p. results in near maximal intensity of proliferated cells.

Reference for mouse: Salic A et al., *Proc Natl Acad Sci USA* **2008**, *105*, 2415-2420.

Reference for rat: Guo J. et al, *Cardiovascular Diabetology* **2012**, *11*, 150.

EdU administration:

- We recommend to perform a single intraperitoneal injection of EdU in PBS (50 mg/kg).
- Euthanize the mouse 4 hours (or longer depending on the cell proliferation duration of the tissue of interest) after EdU injection.
- Remove the desired tissue/organ.
- Snap-freeze the tissue/organ.
- Section the tissue/organ at 20 µm using a cryostat and mount onto appropriate slides.

Cell fixation and permeabilization:

For flow cytometry analysis:

- see step **6**.

For imaging analysis:

- Allow slides containing mounted frozen tissue sections to thaw and warm to room temperature.
- Fix tissue sections with 4% Paraformaldehyde in PBS (pH 7.4) for 15 minutes.
- Wash twice with 3% BSA in PBS (pH 7.4).
- Permeabilize the tissue sections with 0.5% Triton X-100 in PBS for 20 minutes.
- Wash twice with 3% BSA in PBS.

EdU detection:

- see step **7**.

5.2 Nematode (C. elegans):

Reference: Dorsett M, Westlund B, Schedl T, *Genetics* **2009**, *183*, 233-247.

EdU administration:

- Prepare EdU plates: grow MG1693 *Escherichia coli* (thymidine deficient) overnight at 37°C. Dilute the cells 1/50 in 1% glucose, 1.25 µg/mL thiamine, 0.5 µM thymidine, 1 mM $MgSO_4$ and 20 µM EdU in M9 minimal media. Grow this culture at 37 °C for 24 hours in the dark, harvest bacteria and resuspend in a small volume of M9 minimal media. Plate the suspension on 60-mm M9 plates.
- For labelingproliferative cells, place the worms into the EdU plates for 3 hours.
- Wash the plates in PBS to collect the worms and dissect them.

Cell fixation and permeabilization:

For flow cytometry analysis:

- see step **6**.

For imaging analysis:

- Fix sections in 3% Paraformaldehyde in 0.1 M K_2HPO_4 (pH 7.2) for 5 minutes at room temperature.

EdU detection:

- see step **7**.

5.3 Cricket (Gryllus bimaculatus):

Reference: Bando T, Mito T, Maeda Y et al., *Development* **2009**, 1*36*, 2235-2245.

EdU administration:

- We recommend to perform a single intraperitoneal injection of EdU in PBS (50 mg/kg).
- Euthanize the cricket 4 hours after EdU injection.
- Remove the desired tissue/organ.
- Snap-freeze the tissue/organ.
- Section the tissue/organ at 20 µm using a cryostat and mount onto appropriate slides.

Cell fixation and permeabilization:

For flow cytometry analysis:

- see step **6**.

For imaging analysis:

- Allow slides containing mounted frozen tissue sections to thaw and warm to room temperature.
- Fix tissue sections with 4% Paraformaldehyde in PBS (pH 7.4) for 15 minutes.
- Wash twice with 3% BSA in PBS.
- Permeabilize the tissue sections with 0.5% Triton X-100 in PBS for 20 minutes.
- Wash twice with 3% BSA in PBS.

EdU detection:

- see step **7**.

5.4 Chicken (Gallus domesticus):

Reference: Kaiser CL, Kamien AJ, Shah PA, *Laryngoscope* **2009**, *119*, 1770-1775.

EdU administration:

- Perform a single subcutaneous injection of EdU (50 mg/kg) in sterile PBS (pH 7.4).
- Euthanize the bird 8 hours after EdU injection.
- Remove the tissue/organ.
- Snap-freeze the tissue/organ.
- Section the tissue/organ at 20 µm using a cryostat and mount onto appropriate slides.

Cell fixation and permeabilization:

For flow cytometry analysis:

- see step **6**.

For imaging analysis:

- Fix the cells in chilled 4% Paraformaldehyde in PBS for 1 hour.
- Rinse 3 times in PBS for 5 minutes each rinse.

EdU detection:

- see step **7**.

6. Cell fixation and permeabilization for flow cytometry analysis

See also according User Manual

This protocol was developed with a fixation step using 4% Paraformaldehyde in PBS, followed by a saponin-based permeabilization step. The saponin-based permeablization and wash reagent 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.

6.1 Wash the cells with 3 mL of 1% BSA in PBS. Pellet the cells and remove the supernatant.

6.2 Dislodge the cell pellet. Add 100 µL of the fixative solution to the cells. Mix well and incubate for 15 minutes at room temperature. Protect from light.

6.3 Remove the fixation solution and wash the cells with 3 mL of 1% BSA in PBS. Pellet cells and remove the supernatant. Remove all residual blood cell debris and haemoglobin before proceeding.

6.4 Dislodge the cell pellet. Resuspend the cells in 100 µL of 1x saponin-based permeabilization buffer in PBS. Mix well and proceed to step **7** for the click reaction.

7. EdU detection

See according User Manuals

8. Imaging/flow cytometry analysis

See according User Manuals

9. Example of the data derived from an EdU in vivo Kit based experiment:

This EdU *in vivo* kit allows detection of cell proliferation in whole animals, showing no toxic effects. Cell proliferation is detected in different organs by applying the reagents of this kit. In healthy mice cell proliferation is nicely detected in lymphnodes, but not in other organs showing the reliability of this baseclick method.

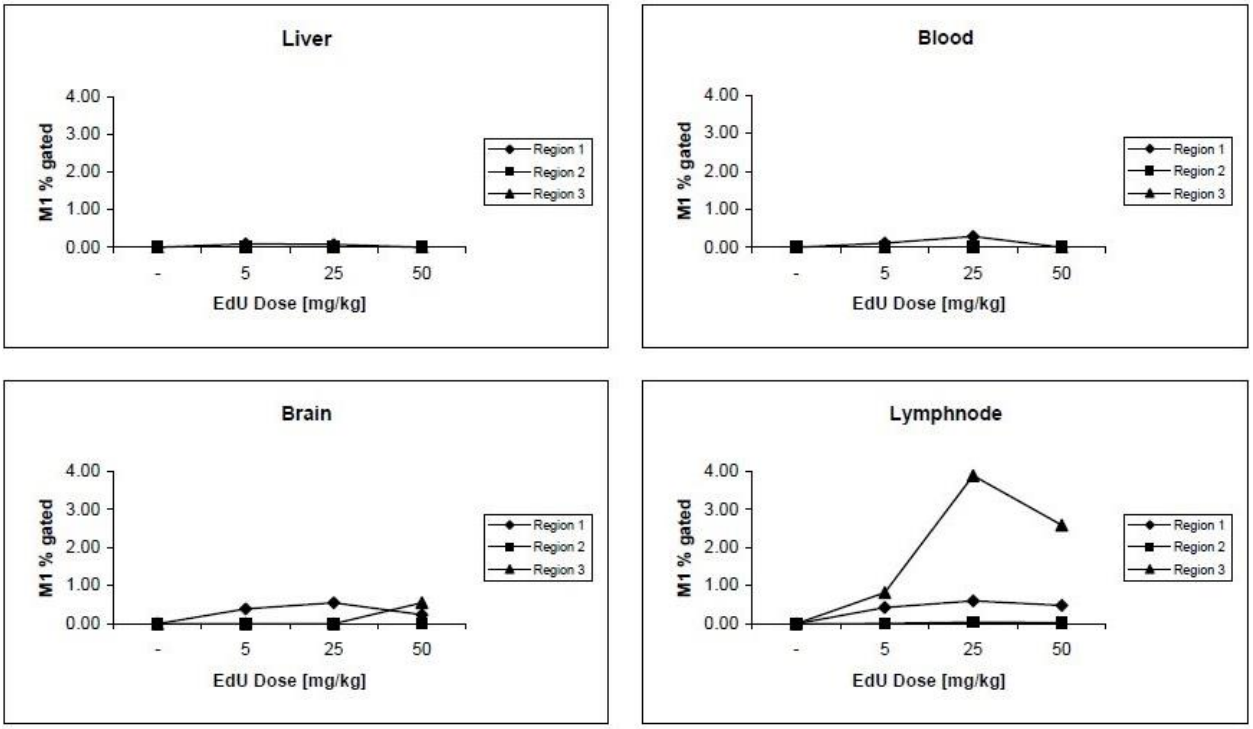

Figure 1: NMRI mice treated with different concentrations of EdU intraperitoneally and sacrificed after 4 hrs. A flow cytometric method is used to analyze the distribution of EdU in different organs.

The shown *in vivo* experiments were performed in collaboration with a third party after having received the allowance of the local authorities.